

Who's playing God now?

Genetic manipulation is in for another bout of examination, even regulation, in the United States, but for the wrong reasons.

FUSS about genetic manipulation has not, after all, gone for good, at least in the United States. Just when people were coming to believe that the Recombinant DNA Advisory Committee (RAC), set up a decade ago by the National Institutes of Health, had finally set public anxiety to rest and might itself ride off into the sunset, a new ruckus has broken out. This time the fellow hurling the chair into the barroom mirror is one Jeremy Rifkin, a long-standing opponent of all genetic manipulation on environmental, economic, social, ethical and even religious grounds (see page 349). Rifkin first made his reputation in this field by disrupting a National Academy meeting on genetic manipulation in 1978 by means of a demonstration in which banners were unfurled equating recombinant DNA research with Nazi eugenics. Now, Rifkin has latched onto the convenient issues presented by proposals to release engineered organisms into the environment (see *Nature*, 2 September, p.262) and by the prospect that deliberate genetic modification of the human germ line may one day be possible. In quick succession, he has generated two lawsuits, an irate letter, a petition and a book which calls for a halt to all such activities. It will be easy to dismiss Rifkin as a rabble-rouser (which he is, or at least would like to be), but it would be mistaken to ignore the serious issues, questions of public safety and ethical dilemmas, underlying this spate of propaganda. Elsewhere, in Australia, Britain and France, for example, there are government committees brooding about the steps that should now be taken and not taken to regulate these activities, but in the United States the President's Commission on the subject has been abolished. Congressman Albert Gore's committee has taken evidence, and may report before too long, but in the meantime there is only RAC to meet whatever needs arise. How well does it fill the bill?

One of the most common complaints is that RAC is merely an advisory group whose decisions and guidelines are binding only on researchers dependent on federal funds. Indeed, RAC advises not the government as such but one of its agencies, the National Institutes of Health, which is not itself a regulatory agency. The result is that members of the public do not enjoy the right to challenge recommendations in the courts as they do in relation to other agencies, the Environmental Protection Agency for example. (Rifkin's lawsuit last week, which complains that RAC had neglected to file an environmental impact statement before sanctioning an experiment in which engineered bacteria would be released into the wild, is obviously flawed in this respect.) But although RAC's guidelines are not binding, the past decade has shown them to have been a powerful influence even on industrial companies simply because a corporation choosing to ignore RAC guidelines or neglecting to seek the committee's advice would be handing powerful and damaging ammunition to all those who might at some future time decide to sue it for damages. As things are, RAC is not merely the nearest entity in sight to a full-fledged regulatory agency, but is effective and acceptable as well.

A second common complaint against RAC is harder to deal with (but not necessarily valid for that reason). Rifkin, in last week's lawsuit, complains that the decision to approve of a small-scale release of engineered bacteria into the environment of northern California was taken by a committee devoid of ecologists. To be fair, RAC did consult outside experts in this field and also the Department of Agriculture's analogous committee, most

probably by now thoroughly imbued with the department's own deep suspicion of all plant infections. But with the shift there has been in the practical interests of the genetic manipulators, there may be a case for a more frequent, even more daring, review of RAC's membership. This does not, however, add up to the conclusion that RAC should be replaced by a *bona fide* regulatory agency of the US government. Explicit regulation would open a can of worms whose consequences RAC's critics seem not to have considered. This is why it is worrying that the Environmental Protection Agency is preparing to issue guidelines governing the general release of genetically engineered organisms on the grounds that recombinant DNA is a "new chemical substance" in the sense of the Toxic Substances Control Act. The agency has not yet disclosed whether it will thus begin to regulate hybrid corn and cross-bred cattle.

The genetic manipulation of human cells raises other difficulties, but fortunately there is time for them to be considered. The most obvious developments, the routine pre-determination of an offspring's sex (not strictly genetic manipulation but selection) and the correction of genetic defects in the somatic cells of people, will not be announced next week or next year (except perhaps in the *National Enquirer*). RAC is moving cautiously to increase its competence in fields such as these — there is talk of recruiting some members qualified to consider the subtle social and ethical problems certain to be posed by genetic manipulation applied to people, especially if germ-line cells are somehow involved. A case can be made for a federal monitoring role such as a strengthened RAC would enjoy. But once people are involved as individuals, it is best that there should be no uniform regulation of the ways in which they can and cannot be dealt with. In the future, as now, it will be necessary that patients themselves, their close relatives, their physicians and the institutions which employ the last should between them decide what should be done in the particular circumstances of each case. Outright bans would deny benefits to some. Uniform rules centrally imposed would invite precisely the threat of government eugenics programmes which the critics rightly say they fear most. □

Financing the poor

It is high time the US Congress put its full support behind IMF.

THE annual meeting of the International Monetary Fund (IMF) in Washington this week is a little like a birthday party without the birthday gifts. The governments that constitute the membership of the fund must decide what rules should govern lending to governments in financial difficulties when the fund's resources are increased from the beginning of 1984. But the United States has so far failed to produce a promise that it will contribute to the planned enlargement of IMF's capacity to lend. The US administration — a late convert to the notion that extra funds are indeed essential — is willing, but Congress has not yet agreed to make the extra credit available. The international importance of the proposed enlargement of IMF's resources has been swamped in a mess of domestic politics, suggesting to the outside world that the United States has no effective mechanism for shouldering the



international financial responsibilities that its financial strength thrusts on it.

IMF is a remarkable institution, designed towards the end of the Second World War as an international bank, a lender of the last resort. The principle is that the fund's resources are provided by governments, which alone are entitled to borrow from the fund. (There is no physical transfer of resources, but only a collection of pieces of paper on which governments agree to contribute in fixed proportions whenever IMF has to make a substantial loan.) For most of the post-war period, the fund's resources have been used to help governments of industrialized countries out of financial difficulties — Britain, for example, has been a frequent borrower. Since 1974, however, the fund has had a wider role to play in helping poor countries lacking oil to stay afloat. In concert with the commercial banks, IMF in the past decade has played a central part in the recycling of the financial surpluses of the oil-exporting states, borrowing from the oil producers and lending on to their potential customers. The trouble is that the flaw in these arrangements has come to the surface. Poor countries that have imported oil with borrowed funds are saddled with a load of debt which they cannot hope quickly to repay.

Except in the US Congress, the seriousness of the problems thus created has been widely appreciated. Three years ago, for example, the Brandt Commission on the plight of the developing countries of the world, argued that only a substantial increase of IMF's capacity to lend to governments in trouble could prevent a further impoverishment of the poorest countries of the world. The objective was not to provide them with further gifts of cash or in kind, but simply with loans that would give them a breathing space and a chance to put their financial affairs in order. In the event, the members of IMF have responded only cautiously to this obvious need. Earlier this year, they decided not to double IMF's capacity to lend, but merely to increase it by a half. For the United States, the largest member of the fund, the extra hypothetical contribution to the fund amounts to \$18,700 million. The Congress is unable to agree that such a sum should be made available in an emergency, and this week's meeting will have broken up long before the issue is decided, one way or the other.

Why should such an obvious need be so casually denied? The arguments are several, and mostly based on misconceptions. The most glaring of these seems to be the persisting confusion that increasing the resources of IMF is tantamount to giving away the sum of money concerned. In reality, however, what is required of the United States is merely the equivalent, on an international scale, of an investment in a domestic commercial bank. Another paradoxical argument is that the proposed increase of IMF's lending capacity will eventually be used to enable governments deeply in debt to the international commercial banks to keep up with their repayments, thus saving important parts of the commercial banking system from bankruptcy. It is, however, mystifying that serious politicians should be prepared to contemplate the bankruptcy of governments overseas and of important financial institutions on their doorsteps simply so as to prove to the banks that much of their past lending (often wished on them by governments) has been unwise. The consequences of that could be the collapse of the international monetary system, and the disruption that would follow everywhere. □

An education crisis

US high schools are not responding to treatment.

WHAT is to be done about public high-school education in the United States? In the air of crisis that has grown up since the appearance last April of the report *A Nation at Risk* from the Commission on Education, only one thing has become clear: teachers' salaries must be increased if well-qualified recruits are to enter the profession — and those now in post persuaded to stay (see *Nature* 25 August p.671). The point is freshly documented in a report published recently by the Carnegie Endowment for the Advancement of Teaching (*The Condition of Teaching — a State*

by State Analysis). Among other things, the report shows how great is the variation of teachers' pay from one state to another (an average of \$33,000 a year in Alaska, a mere \$14,000 in Mississippi), how rapid has been the flight of graduates from careers as teachers and how quickly the drift away from the schools is growing. But more pay is merely a necessary condition for preventing further deterioration in the high school and may never materialize if the central government continues to insist that the states must find the extra money from their own resources. In the circumstances, what conditions would have to be fulfilled if a lasting improvement of high-school education were to be engineered?

The obvious temptation is to go back to the 1950s, when the late Jerrold R. Zacharias was able confidently to call for a "commitment of the American scientific community to enlarge its presence in the classroom". For a time, it seemed as if Zacharias's crusade would succeed. A small army of professional scientists, mostly academics, was indeed drawn into the hugely entertaining process of devising new courses and experiments for high schools. Curriculum development spread from physics to chemistry and biology, while what is still quaintly called the "new" mathematics became firmly established in the schools. Throughout the 1960s, it seemed as if the scientific community was indeed prepared to keep the promise for which Zacharias asked. But then (in 1970) the funds for curriculum development began to shrink — and the outside helpers began to melt away.

Now, with hindsight, it is strange how little tangible evidence remains of that period of great excitement. No doubt confident teachers in the high schools teach more authoritatively than they would otherwise have done, while there has been a great boom in the teaching of biology. But high-school courses in physics, for example, are no more popular now than they were before the sputniks appeared in the sky. Opportunities for teachers of science to improve their skills are far too few. And, with honourable individual exceptions, the links between high-school science and the remainder of the scientific profession have withered away. The problems besetting the teaching of science in US schools are neither an exception (see p.350 for Soviet problems) nor the rule; in Britain, for example, the numbers of students opting for science courses in secondary schools are growing steadily, performance in school-leaving examinations is improving and there may just be enough momentum in the system to ensure that these tendencies continue.

Naturally enough, professional societies in the United States are alarmed at what has happened. The American Institute of Physics has filled the current issue of its house-journal *Physics Today* with a series of contributions designed to demonstrate that physics should be the cornerstone of science education, that physics teachers are too badly paid, that recruitment must be substantially increased and that industrial corporations have an important part to play in revivifying the teaching of science at high-school level, and that it would be possible for people who have retired as active physicists to help out at neighbouring high-schools to everybody's advantage. But if there are resources that might be recruited to the schools, there are probably activities other than the teaching of physics in which they could be profitably employed. Mathematics is a greater need, so too is language learning.

What this implies is that it may be necessary, in a large proportion of high-schools in the United States, to prepare young people for a lifetime in a technical society without being able to offer them an adequate understanding of it. That is an alarming but not disastrous prospect. Mathematics and language are at least the means by which high-school graduates can hope to find out by their own wits what the modern world is like. And that is why, as different school systems in the United States work out their individual responses to the cries of crisis from the centre, they should be dissuaded from solutions of what seems to be the problem which are altogether too literal. It is true that technical skills will be even more important in the future than they are at present, but it does not follow that they should or even can be taught effectively without a thorough foundation of even older skills. □

Recombinant DNA

Rifkin's regulatory revivalism runs riot

Washington

AFTER years of relative calm, the Recombinant DNA Advisory Committee (RAC) of the National Institutes of Health has once again been thrust unwillingly into the headlines. It is being sued for approving tests which release DNA organisms without preparing the environmental risk assessment its critics say is required by law. It is being deluged with Freedom of Information Act requests to uncover alleged conflicts of interest within its membership. And it was subjected last week to some sixties-style activism when Jeremy Rifkin, a veteran opponent of genetic engineering, tried without success to have a court open the doors of a closed meeting at which RAC was deciding whether to approve DNA field tests by Cetus Corporation and Biotechnics International.

Rifkin finds the discomfiture of RAC profoundly satisfying. Since 1977, when he wrote an anti-DNA polemic, *Who Should Play God?*, Rifkin has transformed his dislike of genetic engineering into a single-minded crusade in which the techniques of litigation, persuasion and confrontation are freely mixed. Over the years his tactics have changed but his objective remains the same. In an interview last week he said he would not now repeat his notorious disruption of a National Academy of Sciences meeting when his supporters swarmed onto the stage and unfurled a banner equating genetic engineering with Nazi eugenics. But he continues to believe that all genetic engineering — using classical as well as DNA methods — should be stopped because, in some religious sense, it destroys the sacredness of life.

From his base as president of the Foundation on Economic Trends, a non-profit group in Washington and formerly the People's Business Commission, Rifkin has waged a vigorous campaign to portray genetic engineering as an activity that is, in his own words, "outrageous — an attempt to bring order and predictability to living things that have been spontaneous, disorganized and alive". He finances his campaign through lecturing (most often at small religious colleges at \$2,000 a day) and sales of his many books — a hodgepodge of ambitious tracts which try to fuse scientific and social issues in a manner that makes most professional scientists and philosophers wince, but stimulates large sales. *Entropy*, published in 1980, tries to apply the laws of thermodynamics to economic theory and is a bestseller in Japan. *Agency*, published in May, warns that biotechnologists ("algenists") will alter the

essence of life and try to transform the world into "a perfectly engineered, optimally efficient state".

Because of his awe of the intricacies of nature, Rifkin believes in God, and has succeeded in winning strong support from religious organizations in the United States. In June he persuaded an ecumenical assortment of religious leaders to ask Congress to prohibit experiments that would alter the human germ line (see *Nature* 16 June, p.563). The Church, he says, could become a potent force against genetic engineering because of its huge influence in the United States. Rifkin may also persuade

AIDS research

To review or not to review?

Washington

THE University of California has landed itself in political hot water for insisting that an emergency state appropriation of \$2.9 million for research into acquired immune deficiency syndrome (AIDS) be distributed on the basis of peer review rather than to a pre-selected group of university researchers. The legislator who sponsored the appropriation, California State Assembly Speaker Willy Brown, has joined homosexual activist groups in accusing university administrators of bureaucratic foot-dragging and has suggested that the university's actions may have destroyed its chances of receiving future state appropriations for AIDS research. Meanwhile, several faculty members have accused their colleagues of trying to dodge normal peer-review procedures by having approached the legislature directly for the supplemental funding with the understanding that it would be funnelled directly to them.

With \$1.6 million already allocated, and the remainder to be awarded shortly after 15 October, an uneasy truce has emerged. But worries persist over the effect that the incident will have on the touchy relationship between the university, which under the state constitution is guaranteed autonomy, and the legislature, on which it nonetheless depends for funding.

The idea of the emergency state appropriation first came up, according to Brown's office, at meetings last spring with homosexual activist groups who complained that federal funding for AIDS research was inadequate. In late April, Brown met 28 University of California researchers who have been active in AIDS studies and asked them how much additional support they felt was needed. The figure they arrived at — \$2.9 million — represented the differ-

ence between the support that the researchers had requested from the federal National Institutes of Health (NIH) and the amounts they had actually received.

The university administration then arranged for a quick review of the researcher's projects by an existing university committee, the Cancer Research Coordinating Committee, which met on 23 June and approved 13 of 18 projects submitted. A month and a half later, when the budget was finally approved, the problems began in earnest. While Brown began pressuring the university to provide the researchers he met in April with the full support they had requested, the university insisted that a general request for proposals open to all university researchers had to be issued and fully peer-reviewed. Meanwhile, a new university AIDS task force was appointed and several of its members, learning of the political background of the appropriation and Brown's understanding of how it was to be spent, threatened to resign. But after assuring the panel that it would issue an open call for proposals and conduct a full review of them, the university administration persuaded the members to stay.

Although much of the heat has died down, bad feelings continue to surface over the affair. Recently two of the researchers that met Brown in April and who were not awarded the full amount they had requested, publicly turned down the grants. And a member of Brown's staff, arguing that the pre-selected research group had already had their projects peer-reviewed by NIH, said that the \$1.3 million to be awarded in October is simply being "put up on the auction block". She added: "We don't feel it's too much for the university to react to an emergency by bending some of its rules."

Stephen Budiansky

UN biotechnology centre

India blocks site decision

THE United Nations Industrial Development Organization (UNIDO) has moved one small step towards establishing a biotechnology research centre devoted to Third World needs. In the frequently exasperating manner of UN decisions, the centre now exists — but only on paper. At a meeting in Madrid last week, delegates from 25 nations agreed to set up the centre, but made no decision on where it should go, or who should pay for it despite strong recommendations from an expert committee and some \$150 million on offer from prospective hosts. The decision has now been referred to a new committee, which will report back to UNIDO next year.

The recommendation of the expert committee of biotechnologists from Argentina, China, Hungary, Indonesia, Nigeria, Sweden, Yugoslavia, and Ireland that the centre should go to Brussels, Trieste or Bangkok was not even formally discussed. According to one observer, the impasse was a result of high-level diplomatic pressure from India which has insisted that the centre go to New Delhi.

Most delegates, it seems, had been instructed by their governments to vote for India — but were impressed at the same time by the expert committee's rejection of the New Delhi location.

According to the expert committee, it would be very difficult to set up a regular supply to a centre in New Delhi of fine chemicals, biochemicals, and radioisotope labelled materials which must be "obtain-

ed on a day-to-day basis from international suppliers". The supplies would be hindered by currency and customs restrictions in India, the committee felt.

Also, said the committee, there was little genetic engineering *per se* in India — probably because the difficulties of working in India have made it impossible to attract and repatriate Indian genetic engineers abroad. There is good molecular biology in the country (notably in Bangalore), according to the committee, but these centres are far from New Delhi. The committee was thus concerned that a biotechnology research centre in New Delhi would suffer from isolation. These conditions did not apply in Bangkok, they said, and even less in Brussels or Trieste.

However, India argues that the right environment could be supplied in New Delhi; that their expatriate experts would come back if the centre was established in India; and that if the centre were in a developed country it would suffer from a different kind of isolation: academicism, and isolation from Third World needs.

In Madrid, the compromise and temporary solution was to "establish" the centre but hold no vote. The past expert committee was non-governmental, and to that extent apolitical and unbiased. The new committee to advise on siting will be governmental. As a result the UNIDO biotechnology centre is likely to be even more politically exposed than before.

Robert Walgate

India's bait for expatriates

New Delhi

THE continuing efforts of the Indian Government to encourage expatriate scientists and doctors to return home seem to have backfired. Far from being pleased to welcome back their colleagues, scientists that stayed at home are complaining that those responsible for the "brain drain" are being promised better treatment than those who loyally resisted the temptations of high salaries in the United States and elsewhere.

Prime Minister Indira Gandhi announced the latest proposals last month — the government intends to set up a new "technology city" where Indian scientists now working abroad would be able to work in the sort of conditions that they have become used to. Research areas for the town would be in "frontier sciences" such as space, fibre optics and lasers, and the cost of the project is estimated at US \$125 million for the infrastructure and first class laboratory equipment.

The government has been trying for some years to stem the flow of highly qualified scientists and technical personnel to the developed countries. A study by the United Nations Conference on Trade and

Development estimated that India loses the equivalent of US\$17,200 in education costs for every scientist leaving the country and US \$33,000 for every doctor.

So far attempts to reverse the trend have met with little success. The United Nations-financed TOKTEN programme (for Transfer of Knowhow Through the Expatriate Nationals), which is intended to bring back expatriates for brief periods of a few months, has also evoked little response. Mrs Gandhi seems to be hoping that the technology city will help bridge the gap.

Plans for the city have not yet been finalized but initial reactions have been hostile. Professor M.V. Hariharan, dean of research and development at Bombay Indian Institute of Technology, says that top scientists in India whose sense of commitment leads them to stay despite poor research facilities would be penalized. "Why not build a science city for them?" he asks. Others point out that by "pampering" returning scientists the government runs the risk of creating two classes of scientists working in the same field — one privileged and one making do with second best.

Sunil Saraf

Soviet science teaching

New moves for more scientists

SOVIET universities and technical schools have had to lower their admission requirements in order to fill the less popular courses, the Minister of Higher and Special Secondary Education, Dr Vyacheslav Elyutin, has admitted. Although all this year's one million university places were eventually filled, this was achieved only after reducing the entrance examination average mark from 4.5 (on a 5-point scale) to 4, and "much vocational guidance" of the applicants.

Although the minister did not say which were the less popular courses, according to the rector of the Moscow Energy Institute, Dr Valentin Grigor'ev, the shortfall in science and technology applications is "alarming" — over the past decade, there has been a 20 per cent drop in applications. Much of the blame, Dr Grigor'ev suggests, lies with "incorrectly organized" vocational guidance in schools, which fails to overcome "materialist attitudes" in the young people's home and school background.

There is a general feeling that the pure sciences carry more prestige than applied, but there have been no suggestions that material gains are less for technologists than for academics. By "materialist attitudes" Dr Grigor'ev may have been referring to the problems arising from the rule that Soviet graduates have to work for three years in a post assigned to them, which can be anywhere in the Soviet Union. Engineers and technical specialists are all too likely to be sent to the remoter areas of Siberia and the Far North, where conditions are primitive. During the past few years, there have been frequent complaints that too many new graduates are claiming exemption on health or compassionate grounds from taking up appointments in remote areas, while some manage to "disappear" each year, in spite of the strict system of internal passports.

New planning procedures are therefore being introduced to ensure the necessary supply of technical graduates not only in absolute numbers but also in the right places. In the developing regions of Siberia, the Far North and the Far East, admissions to local universities and colleges have been increased, while universities in the European regions of the Soviet Union are sometimes taking "special admissions" from the developing regions, on the understanding that the young people so trained will return home when they graduate.

At the same time, new science curricula have been introduced throughout the Soviet Union, with a greater emphasis on "foundation" theoretical training, less fragmentation of courses and, in particular, the integration of mathematics into the general syllabus.

Vera Rich

Windscale 1957 accident

Polonium not a hazard?

EVEN when release of polonium-210 is taken into account, less than 33 deaths will be caused by radioisotopes released during the 1957 fire in a reactor producing materials for nuclear weapons at Windscale, North-West England. So concludes the National Radiological Protection Board (NRPB) in its revised assessment of the consequences of the fire, published this week in response to a claim that the number of deaths had been grossly underestimated because NRPB had forgotten to take polonium release into account. The revised assessment of population dosage, 2,000 man-sieverts, is an increase of 67 per cent on the earlier estimate, still too small for health effects to be detectable.

At the time of the fire, which burned for two days, polonium for weapons use was being manufactured in No. 1 pile by irradiating bismuth. Tritium was also being produced. Official investigations of the fire considered only the fuel fission products that were released, and NRPB's original assessment was based on those reports (see *Nature*, 7 April, p.470). The revision was prompted by an article by John Urquhart, of the University of Newcastle, published earlier this year in *New Scientist*. Urquhart claimed that the inclusion of polonium in the assessment would increase estimated population dosage several hundred times and suggested that around 1,000 deaths would result from the fire.

Urquhart's article produced some red faces at NRPB, since there are references to the polonium released in the fire in the open scientific literature. For its revision NRPB has been given access to several studies on the fire that have hitherto been classified. Five nuclides not considered in the earlier report are examined, though only polonium-210 makes a significant contribution to effective population dosage. The inclusion of polonium leads to a figure of 33 for the estimated number of "health effects" (fatal cancers and hereditary defects), compared to 20 in the earlier assessment. It is, however, stressed that these figures are upper limits, because they assume a linear relation between dosage and mortality. The revision also reveals that 135,000 curies of tritium and some plutonium were released.

The discrepancy between NRPB's new conclusions and the estimates of Urquhart are due mainly to their different models of polonium transport in the environment; Urquhart based his calculations on an earlier NRPB study that has also since been revised. The increase in the number of health effects which follows from NRPB's revision is within the range of accuracy claimed in NRPB's initial assessment. Urquhart is still not happy, however: measurements in 1957 of alpha activity in milk published by, *inter alia*, NRPB's

director, Mr H J Dunster, show levels that are 500 times greater than the revised assessment predicts. This inconsistency is dismissed by NRPB as "spurious". Urquhart says that NRPB is "rewriting history": as only two measurements were made of alpha activity in foodstuffs near Windscale, there is no more reason to reject Dunster's figures than any other. Urquhart called on Dunster to resign unless NRPB can explain why the published results of its own director were dismissed in favour of another measurement that fits

US nuclear safety

Who-does-what dispute

Washington

A LAWSUIT filed by US environmentalist groups last week has exposed an embarrassing jurisdictional argument between the Department of Energy (DOE) and the Environment Protection Agency (EPA) over who should be responsible for environmental health and safety at nuclear research facilities.

The suit, brought by the Natural Resources Development Council (NRDC), challenges a long-standing claim that DOE nuclear facilities are exempt from the provisions of the Resource Conservation and Recovery Act (RCRA), which regulates the storage and disposal of hazardous wastes. The EPA, which administers RCRA, has also challenged DOE's exemption — although it is trying to change DOE's mind by persuasion and not by litigation.

What has brought the issue to the surface is mounting criticism of the way hazardous wastes have been spilled by DOE's Y-12 plant at Oak Ridge, Tennessee. Operated for the department by Union Carbide, Y-12 is primarily a production facility for nuclear weapons components. Inquiries by the Tennessee department of health and environment and by a congressional committee have resulted in evidence of widespread pollution by Y-12 and the "loss" of some 2.4 million pounds of mercury.

Most of the discharge of mercury apparently took place between 20 and 30 years ago, and the process that used mercury was stopped in the 1960s. But mercury is believed still to leak into a creek that flows through an adjacent neighbourhood, and NRDC says that Y-12 still disposes "haphazardly" of other toxic substances which are contaminating ground water and creeks that flow into the Clinch River.

The Tennessee health and environment department has persuaded Oak Ridge to agree to cooperate in a long-term plan to clean up the site and reform waste management precautions. As a first step, the plant will close four unlined waste disposal ponds which, according to the state authorities, have caused leakage of hazar-

more closely with models of polonium transport used in the assessment.

Dr Gordon Linsley, who is co-author of the NRPB assessments, replies by pointing to other inconsistencies in Dunster's data and says there are independent reasons for believing them to be wrong by at least two orders of magnitude: it could be simply that the units are incorrectly specified. Linsley acknowledged that Urquhart had performed a public service in drawing attention to polonium but is disturbed that he has still not presented detailed calculations for peer review in a recognized scientific journal. Urquhart's plan is to publish his work in a new journal to be edited by himself.

Tim Beardsley

dous substances into the headwaters of a nearby creek.

The NRDC, however, does not believe that by agreeing to some of the remedies proposed by the state DOE has gone far enough. It wants a court to confirm that Y-12 is in violation of the Clean Water Act and the RCRA regulations, so that the remedies can be legally enforced and the RCRA provisions applied to other nuclear facilities for which the DOE has claimed exemption.

It will be far from easy to resolve the legal issues. Although Congress clearly intended RCRA to apply to all federal agencies, the act says that its provisions can be waived when they conflict with activities authorized under the Atomic Energy Act — activities that include virtually all nuclear research production. DOE argues that applying RCRA to its facilities would duplicate the department's own regulations, while NRDC claims that RCRA would strengthen, not conflict with them.

One ironic consequence of the NRDC suit may, however, be to force EPA and DOE to close ranks. At present, EPA would like to see RCRA applied to non-radioactive and some mixed waste produced by DOE plants, while leaving DOE in complete control of radioactive wastes. The prospect of a court hearing with public attention focused on differences between the two agencies, is likely to hasten moves towards an agreement between them.

Meanwhile, Congress will join the debate on Oak Ridge next month when Representative Albert Gore's investigations and oversight subcommittee issues a report on an investigation into pollution at the plant. Gore, a Tennessee Democrat, is more concerned about cleaning up Oak Ridge than he is about the jurisdictional argument between EPA and DOE. His committee is therefore expected to recommend the appointment of an external scientific panel to oversee remedies at the plant but remain silent on RCRA.

Peter David

Bell Labs

New order augers well

Murray Hill, New Jersey

BELL Labs insists that its fundamental research will not be harmed by the upheaval in the affairs of its parent companies due to take effect on 1 January. But even at this late stage, the Federal Communications Commission (FCC) has not yet agreed the details of how the cost of the largest private research organization in the world is to be met.

Dr Arno Penzias, the director of the basic research division (affectionately known as Area Eleven), is unabashed. As evidence that basic research will survive more or less intact, he says that his biggest preoccupation just now is recruitment. His division is to lose 100 of its 1,000 people on 1 January, and he hopes to make good the loss by skimming the cream from this season's crop of graduates, mostly at the doctoral level.

The coming upheaval is the impending break-up of the telephone company AT&T, decreed last year by the courts in settlement of the suit against the telephone company brought by the US Department of Justice. The question of Bell Labs' future was sharpened during the court proceedings by the frequent statement by AT&T officials that any change would put fundamental research at risk.

Penzias says that the only damage done so far is to his own research. "Last year, I published only one paper, and that was work I should have written up earlier." The snag is that he took over Area Eleven only a few weeks before the court settlement. Since then there has been no time for astrophysics: he holds a Nobel prize for work on the microwave background radiation.

For the future, he relies to a large extent on the promises which have been made by AT&T to Bell Labs and by the internal

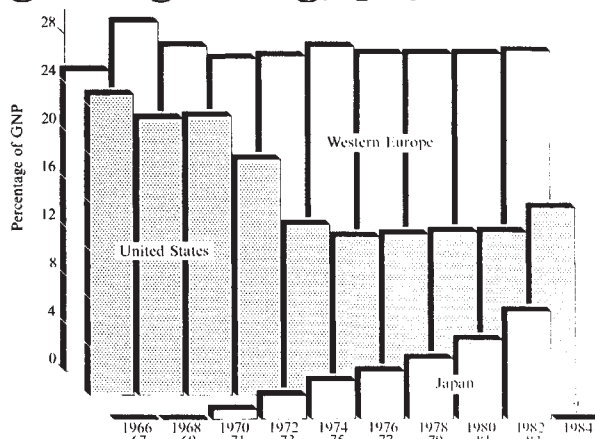
understanding he has reached about the importance of his own patch. For Bell Labs as a whole, the staff will shrink from 26,000 to 18,000, and the budget from \$2,100 million to \$1,800 million, but Area Eleven still plans to have \$200 million to spend.

For Bell Labs as a whole, the January upheaval will be a bigger trauma. For the time being, 7,000 of the 8,000 people being shed will remain on this site, 4,000 of them working for the new Central Services Organization intended to provide research and development services for the seven regional networks into which the peripheral parts of the network will be divided in January. A further 3,000 people will work for the new central information services company, the part of AT&T that will in future be allowed to compete with other organizations in data processing of various kinds, videotext services for example.

Bell Labs will be financed in an entirely novel way. Hitherto, its funds have come from a 1 per cent tax on the revenues of the 22 companies operating local networks within the Bell system, but the inheritors of those companies will in future pay only for the cost of the Central Services Organization. What is left of Bell Labs will be supported by the four centralized operating companies (the trunk network, manufacturing facilities and operations overseas and the new information systems company).

Penzias says he is satisfied with the formula (which he calls an algorithm) so far worked out for "taxing" the four operating companies. The principle is that each company should contribute towards the joint cost of AT&T's rump of a headquarters and of Bell Labs according to a four-component measure of the scale of its operations. FCC is still unhappy with some of the details, however.

Spending on high energy physics



The ups and downs of spending on high energy physics research as a percentage of gross national product (GNP) for Western Europe (the member countries of CERN), the United States and Japan have recently been calculated by Professor Tetsuji Nishikawa, head of Japan's new high energy physics research laboratory (see p.379). (Except for 1984, figures are two-year averages — no figures are yet available for spending in Japan in 1984).

No fellow feeling

RECENTLY-elected Fellow of the Royal Society, Mrs Margaret Thatcher, is being made unwelcome by a group of 44, anonymous, fellow Fellows who have written to Sir Andrew Huxley, President of the Royal Society, to protest at her election in June, under "rule 12". Under this rule, candidates may be proposed by Council without the usual paperwork (list of publications and so on), if the individual is thought to have made a contribution to science that is a little out of the ordinary.

The errant 44 would agree that Mrs Thatcher's contribution was out of the ordinary, but not in quite the way that the drafters of the constitution had in mind. They write that it was "damaging to the good name of the Royal Society to propose Mrs Thatcher, whose Cabinet is responsible for highly contentious policies which have had, and are having, very serious effects on education and research".

Nevertheless, in the society's election in June, Mrs Thatcher won the necessary two-thirds majority of the 100 fellows present. And since Mrs Thatcher is now a Fellow, there is little the 44 can do about it, except demonstrate at her admission ceremony.

But the letter may create pressure to have rule 12 changed, to distinguish political appointees (like Churchill, Macmillan and Wilson) from more scientific rule 12 FRSS (like Sir Karl Popper). This question may now find its way onto the agenda of the next Council meeting (on 13 October), a Royal Society spokesman said. Robert Walgate

Penzias is sanguine about the future, arguing that even the huge cost of Bell Labs is only small for an organization whose earnings (trading profit) amount, to \$1000 million a week. Nor does Penzias see a change in patent policy, under which Bell Labs has been required since 1956 to provide non-exclusive licences to all who ask for them and who are willing to pay a royalty. Astonishingly, according to Penzias, there is an agreement with IBM under which the parties provide royalty-free licences to each other on all patents — and reckon not to apply for patents on their most treasured secrets.

In the long run, however, next January's divestiture must bring a change. The whole objective has been to put the separate part of the telephone company on a basis that would make fair competition possible, which means that Bell Labs will depend for its survival not merely on its own cleverness but on the success of the central operating companies, any one of which might in principle be a failure. This no doubt explains how Penzias, two years ago a simple astrophysicist, is now full of the commercial merits of AT&T's new digital switching system, developed at Bell Labs. There will be many more entrepreneurs in Area Eleven before the divestiture is out. □

UK venture capital

Golden opportunities?

UNIVERSITIES and research councils in Britain are waiting to hear what new arrangements the government has in mind for the commercial exploitation of publicly-funded research following its decision to remove the first refusal rights of the British Technology Group (BTG) on development of novel ideas arising from holders of research council grants and research council employees. Whatever happens, it is bound to give inventors and their employers much greater say than hitherto in how exploitation is to proceed, but will also bring increased responsibilities. Many universities will find their new rôle as entrepreneurs unfamiliar, and the government is now devising safeguards to ensure that potentially valuable results do not fall between several stools. Until the new arrangements are clear, BTG considers itself in business as usual.

The first problem is that of protection. Under the 1977 Patent Act almost any public disclosure of an invention (with the exception of international exhibitions) would invalidate a subsequent patent application. The National Research Development Corporation, now part of BTG, is generally agreed to have done a good job on this score, often managing to file applications only days or hours before publication. Many in the universities hope and expect BTG to continue that service, but BTG's present constitution does not allow it to offer a patenting service on a straightforward commercial basis.

A second area for concern is whether venture capital funds will be available for ideas, patentable or otherwise, at a sufficiently early stage. Venture capital funds, which offer long-term equity finance backed up by active management participation, have been thriving in Britain in recent years. According to Ms Susan Lloyd, editor of *UK Venture Capital Journal*, more than 45 independent funds have been established in Britain since 1980 even excluding funds managed by clearing banks and insurance companies. Several companies boast special expertise in new technologies — for example, Advent Ltd, Prutech Ltd and Thompson Clive Ltd. But most venture capital companies will want to see a prototype and a business plan before they invest, whereas BTG have been willing to invest in "a spot on a chromatogram".

If the risks of investment in new technology are high, so too are the potential rewards. Mr David Cooksey, managing director of Advent Ltd, says he looks for returns of 40 per cent compound interest per annum, but he has to be prepared to wait 5 to 10 years, or even longer: investors cannot expect annual dividends. This type of financing has been made possible by the creation in 1980 of the unlisted securities market, where shares in companies not

meeting the strict requirements of the stock exchange can be traded. The government's New Business Expansion Scheme offers generous tax incentives to investors in growing businesses, and available funds seem likely to increase. The biggest problem is in matching up an academic inventor with suitable business expertise. In this respect Britain lags behind the United States where enlightened policies of the Massachusetts Institute of Technology and Stanford are widely held to have been responsible for Route 128 and Silicon Valley.

Another looming problem arises from the fact that while BTG held the purse strings, British inventors were routinely rewarded through their universities or research coun-

cils, often on a formula basis; without BTG individual stakes will be larger and there may be more arguments over ownership.

So what does a venture capitalist like Cooksey have to say to academic inventors? The academic aspiring to be an entrepreneur does not have to give up the university life altogether and he should be prepared to tout his wares to several companies before finding a partner. He may have a lot to gain, and should ensure that his host institution takes its responsibilities seriously, both in the facilities it provides to start-up companies and its investment policies. Some universities, including those at Oxford and Cambridge, have already made investments in venture capital companies; as government provision for universities decreases in real terms there will be pressure on many others to follow suit.

Tim Beardsley

Small-business research

Innovation fund oversubscribed

Washington

THE federal government has been inundated with proposals from small firms seeking research contracts under the Reagan Administration's controversial new law compelling 11 major agencies to spend more of their research money on companies with fewer than 500 employees.

The law, the Small Business Innovation Development Act, was approved by Congress last year over the vigorous objections of the universities, which complained that it would divert scarce federal funds from urgently needed basic research to applied research of dubious calibre. Since then, the Small Business Administration has been waiting anxiously to discover whether small businesses would be able to put forward enough good research ideas to justify spending the \$40 million available under the scheme this year.

To the undisguised relief of the administration, federal agencies participating in the scheme have by now received some 9,000 proposals — more than enough, according to the Small Business Administration, to ensure that the 700 contracts awarded this year will be of high quality.

The act is being phased in over five years and the proportion of federal research funds set aside for small businesses is to be increased annually. Ultimately, it is to reach a level at which every federal agency with an extramural research and development budget of more than \$100 million will set aside 1.25 per cent of that budget for contracts with small firms. As a result, an estimated \$1,400 million of federal research money will be pumped into small companies.

This autumn's first round of contracts will be on a small scale, ranging from \$30,000 to \$50,000 and involving only a few months of work to investigate the technical

feasibility of research proposals. The sponsoring agencies will face harder decisions in the second phase of the exercise, when it will have to decide which projects should be continued and enlarged within a ceiling of \$500,000.

However, controversy about the working of the act persists. Michigan Democrat John Dingell is sponsoring an



amendment in Congress that would prevent the National Institutes of Health (NIH), one of the agencies involved in the scheme, from supporting research proposals that would have failed under normal NIH procedures. Digell fears that the law will result in NIH funds being "raided" to support ideological objectives secondary to the institutes' biomedical research missions. An NIH spokesman conceded that the 598 proposals received under the scheme had achieved lower than average merit scores from NIH reviewers. But she said NIH intended to award its full quota of \$6 million for small business research this year.

Peter David

Franklin Valley not so special?

SIR — With the proposal to construct a dam that would flood the Franklin Valley in Tasmania, the archaeological content of the Franklin Valley caves received wide publicity (*Nature* 300, 679; 1982). Undoubtedly the finds make a valuable contribution to the study of prehistory, but in the context of the debate the perspective became distorted.

In depositions to the High Court in support of the Commonwealth's case it was claimed that the finds were unique and in one declaration Dr Rhys Jones asserted that "it is most unlikely that sites providing material of comparable archaeological value will be found in south west Tasmania outside the (Lower Franklin and Middle Gordon) limestone belts".

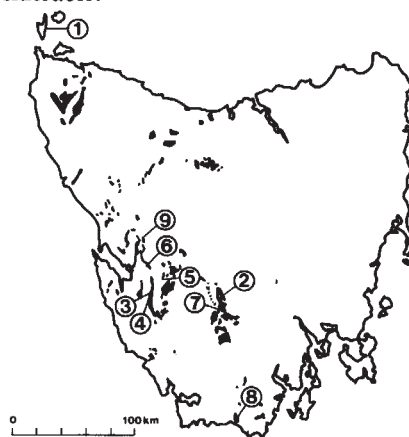
To test these statements and to assess their objectivity, the Hydro-Electric Commission's Geological Section conducted a search for similar caves outside the reservoir area during a two-week period in May 1983. Those conducting the search were convinced that the assertions were wrong, because the potential cave forming rock formations, the Precambrian, Ordovician and Devonian dolomites and limestones are widely distributed throughout the western half of the state. These formations occur in the broad valleys that lie between parallel ridges that are the dominant feature of the landscape. The valleys would have been readily accessible to Pleistocene man, during the period of interest some 20,000 years ago, when they were covered by grasslands or sclerophyll heath and sedgelands, and largely devoid of trees. Today these valleys are covered by dense rainforest and exploration is difficult.

We found five caves, with content similar to the Franklin Valley caves and considered to have the same archaeological significance, in widely separated areas. These caves are in the Andrew, Acheron, Florentine, Nelson and New River valleys. Given that the inland area was abandoned as a habitat about 15,000 years ago (K. Kiernan *et al.* *Nature* 301, 28-32; 1983), it is reasonable to assume that the caves were occupied during the same period as the Franklin Valley caves. The archaeological evidence in the new caves includes large quantities of bones and teeth, split and burnt bones, charcoal fragments, stone artefacts and in one cave a flake of Darwin glass. An additional fifteen caves of possible archaeological significance were located.

The new caves have not been inspected by an archaeologist, because the Association of Consulting Archaeologists Incorporated advised its members not to take part in any recovery operation. Nevertheless, we are convinced of the worth and status of the new finds.

Archaeological investigations in the Lower Gordon area have so far concen-

trated on the proposed storage area to the almost total exclusion of other potentially fruitful areas of search. Our cave study for instance revealed that there are over 1,000 known caves in the extensive cavernous formations, and in the coastal and midland sandstone formations. Very few of these have been inspected by an archaeologist. As a specific example, of the 335 known caves in the Florentine Valley, which is outside the Gordon reservoir area, only about 20 have been visited by an archaeologist. The scope for further research is considerable.



RECORDED PLEISTOCENE CAVE SITE
Radiocarbon Age, Years BP,
(Flood 1983 & Harris, in press)

① Cave Bay - Hunter Island	22 750 ± 420	⑤ Cardia - Acheron Valley
② Beginners Luck - Florentine Valley	20 650 ± 1790	⑥ Lugra - Andrew Valley
③ Kutikine (Frazer) - Franklin Valley	19 750 ± 840	⑦ Nanwoon - Florentine Valley
④ Deenarrena - Franklin Valley	c. 20 000	⑧ Peiniak - New Valley
		⑨ Nelson River Cave



Distribution of cave-forming limestone and/or dolomite of Devonian, Ordovician and Precambrian age.

In another submission to the High Court, Professor John Mulvaney asserted that "the region promises to be a veritable laboratory for research into the society of early *Homo sapiens*, no less than the upper Palaeolithic sites in the Vézère valley in the French Dordogne, has proved for studies of European cultural origins." That the southwest has an archaeological content is not in question, but one does question the perspective. The caves of the Dordogne, the Lascaux Caves, are truly cultural treasure houses. It is generally agreed that they are special caves that were not used for general habitation, and in fact they contain remarkably few implements and animal remains. Archaeologists consider that these caves were used for religious activities, which produced magnificent examples of prehistoric art, with paintings and engravings of animals and vestiges of animals in colours that are fine, clear and vivid today. By contrast, the Franklin Valley caves merely record occupation by hunting parties, and so far no evidence of art or cultural activity has been found.

The proper perspective is that the Franklin Caves are only a few of a number

of similar caves that were occupied by early man in the southwest. In spite of this, archaeologists maintained that the Franklin Caves and their deposits were of such uniqueness and cultural value that only total preservation *in situ* was acceptable. This argument was part of the successful campaign to stop the power project. Clearly the attitude adopted lacked scientific objectivity and emphasizes the wide gap between truth and verisimilitude when science is subordinated to promoting a course.

The social and economic cost of influencing public opinion are obviously great. Therefore, archaeologists can only continue to receive public support if the information the community receives is scientifically correct and sustainable. There must be a balance between the legitimate desire of archaeologists to preserve the relics *in situ* and the development needs of society, which can specify that some relics be preserved by undisturbed recovery. To achieve this end all groups must work together in a spirit of scientific cooperation.

S.J. PATERSON

Chief Geologist,
Hydro-Electric Commission,
Hobart, Tasmania, Australia

Not as it seems!

SIR — As an "occasional" cricketer who has been distinctly awestruck by the movement of the ball in the air — especially in damp conditions — I am disappointed that R. D. Mehta *et al.* (*Nature* 303, 787; 1983) were unable to account for the phenomenon.

One possible explanation is a temperature differential induced by the bowler through rubbing one side of the ball. This hemisphere apparently becomes quite hot and may provide an extra asymmetrical pressure on the ball through the localized effect of the heated hemisphere on the laminar flow of air over the ball, thus the ball would mimic an aerofoil. Perhaps this effect is in some way enhanced in damp conditions.

Mehta *et al.* may like to consider this aspect in addition to their estimation of the impact of the seam itself on the ball movement. Especially as the main weakness of their hypothesis is that it offers as an explanation of the problem of extra movement in humid conditions the claim that it is either enhanced backspin increased by damp conditions improving the bowler's grip (a seemingly weak explanation as humid conditions could increase sweating and reduce the bowler's grip), or that increased swing in humid conditions is an optical illusion. If the latter explanation is to be upheld, surely they should at least provide some indication as to how this illusion occurs, as it is apparently widespread.

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What magic formula for success?

Japan's success in world markets is the envy of the West. Where does science come in the success story?

How and why has Japan succeeded so well? And where will it end? Those are the questions that send people from the West flocking to Tokyo and, occasionally, even into the hinterland. The motive is mostly economic, for in the past decade most industrialized countries in Western Europe and North America have been frightened out of their wits by the apparently endless stream of technical innovation in consumer electronics from Japan, put on sale at prices competitive with and often lower than the bare costs of manufacture elsewhere. Motor cars and cheap high-quality steel (still flowing from Japanese production lines) may have kept politicians and others awake at night, but were not so obviously the products of advanced technology. So is the torch of progress, the leadership of the free world or some other such figurative emblem, about to pass to the Western Pacific? That is why there has been such a surge of interest in Japanese science and technology. And that explains why journalists from the West now compete with delegations of businessmen and government officials bent on begging the Ministry of International Trade and In-

dustry (MITI) to organize a voluntary export quota on the latest electronic product and even, still, motor cars.

Nature has made this fashionable pilgrimage for a somewhat different and more self-interested purpose. Readers know that many of the journal's contributors work in Japan, but may not be aware that close on ten per cent of each week's print-run goes there, now (at last) by air. So Japanese science is as much a part of a single constituency as, in a wider sense, science itself cannot be partitioned by national boundaries. If, as reports suggest, Japanese practitioners have worked some kind of miracle, they deserve congratulations, while other readers may wish to know in more detail what they are about. This is the spirit in which an earlier survey of science in Japan was carried out (see *Nature* 240, 185-220; 1972). Many of the conclusions then (see next page) remain unaltered.

Briefly, the most vivid impression of basic science in Japan is what everybody would expect: some is exciting, some worthy and some downright pedestrian. This was the case ten years ago, as it always has

been elsewhere. Nobody should be surprised at that. In 1972, however, it seemed proper to complain that the government of Japan was shockingly neglecting the needs of university researchers. Resources are now improved, but only patchily; and the government is still unwisely mean (see page 357).

Technology is different. Optimism abounds. The small army of engineers graduating each year from the universities seems uniformly to believe there are no obstacles between feasibility and production. Success feeds on itself, and has spread outside the technical community. Casual acquaintances ask for admiration of "our technology". And well they might, for it has kept them afloat since the oil shock exactly ten years ago. And city crowds at least are visibly more prosperous. But not everything the engineers have touched has turned to export earnings. Even so, there is no obvious reason within technology itself why fields now strong — electronics for example — should not be further strengthened, leading to what has quaintly been called "world domination" in some areas. "So what?" is the only level-headed reply. As the following pages show, Japanese technology is not strong across the board, while the benefits of a rational division of labour between nations are as real as when Adam Smith defined them (intranationally) 250 years ago. And anything might go wrong. In basic science, the uncertainties are intrinsic; defining problems is one battle, solving them another, but then the job is done. In technology, the most worrying uncertainties are extrinsic — political and economic stability, social change and mere accident. That Sakhalin Island (once Japanese) turns out to be so close will give many people pause.

Both Japan and its competitors therefore need to understand the reasons for the successes of the past few years. A few Western myths are easily disposed of. The belief that Japanese technology rides on the back of cheap labour is belied both by the statistics on wages in manufacturing industry (but young people do less well than in the West) and by the continuous improvement of labour productivity, more than 8 per cent a year since 1960 or three times as fast as in the United States. The charge that Japanese successes are simply imitations of Western innovation is similarly mistaken. Much technological innovation succeeds if it leads to an improvement of only a few per cent in some perfor-

Accreditation

THE following pages have been compiled and written by Alun Anderson and John Maddox, of whom the former speaks a little Japanese. The other considers the following observations relevant.

Nobody ignorant of the language can be certain that he is gathering information accurately, or that he has understood why its source considers it relevant. But the cheerfulness and confidence of people in government, industry and the universities suggests that people mostly know what questions they are answering. The big handicap is not being able to read the documents that people give you freely.

Japanese was clearly designed to be almost but not quite impossible to learn. Why else would a set of ideographs borrowed from the Chinese (with 3,000 or so in common use) be embedded in a perfectly good 50-symbol alphabet, with an entirely separate alphabet for foreign words?

The Japanese, however, are fond of wondering whether the sheer difficulty of it all may be a valuable training for the mind, perhaps even an explanation of the recent suggestion (R. Lynn, *Nature* 292, 222; 1982) that IQ is higher in Japan than

elsewhere. One consequence, however, is that since typing is virtually impossible, the usual office traffic in memoranda is only a trickle, the handwritten note and the photocopy are a poor substitute, so that office workers sit as they have always done — up to a score of them in a smallish room, within earshot of each other. The system saves rent but may be swept away with the appearance on the market of word processors able to accept phonetic (even latinized) input and concert it into the proper jumble of Chinese and Japanese characters.

In the past ten years, many more Japanese have learned English (the second language for at least four high-school years). At a guess, getting on for a half of younger academic scientists are reasonably fluent. One snag is that in more formal settings, English-speakers will not reveal themselves if a superior's lack of skill might then be revealed.

So may it soon be that language will cease to be a problem? A friend, a successful businessman, took this speculation badly, thinking it worth a homily on the theme "Why should we give up such a beautiful language?" in which the word colonialist appeared. □

mance characteristic. Japanese companies do not disparage such innovations, but see them as a way to please customers, increase sales and perhaps make a fortune. They have paid for patent licences like men. Perhaps manufacturers in the West should follow suit. But who can say that the electrostatic microphone or the device that allows joggers to hear Beethoven's Ninth while running are anything but ingenious exploitations of a market?

The biggest Western myth is that the boom has been engineered by MITI, supposed for these purposes to be an omnipotent manipulator of companies' intentions, not to mention the value of the yen on the foreign exchanges. The ministry is, on paper at least, no more influential than, say, the Department of Commerce in the United States. What MITI does is to spend modest sums of its own money on stimulating collaborative research projects by industrial companies, stopping short at the awkward stage where exploitation is on the cards. (The cost of the much-vaunted fifth generation computer at ¥23,000 million over eight years is not very different from the £400 million spent over five years in Britain alone on collaborative research.) Japanese companies roar with laughter when it is suggested that MITI has an influence on the pattern of their development. The need to compete with each other is too urgent to wait on the bureaucratic timetable.

But MITI can be blamed for its part in the non-tariff barriers to trade of which Japan's trading partners loudly complain. Tariffs, entry taxes on imports, are not very different from those elsewhere, while the complainants agree that many of their past failures in the Japanese market owe much to its complexity and obsession with quality. But there is also a system whereby imports are inspected, consignment by consignment, for compliance with Japanese quality and safety standards as they arrive. There is also a system of import quotas, chiefly for agricultural products, which has had the United States for years complaining that Texas could supply the whole of Japan with beef if only the trade were free. The complaint ignores the political difficulties any Japanese government would face in deciding to put its farmers out of the beef business.

Complaints about export prices are more substantial, but again may stem from ignorance. The dependence of Japanese manufacturers for their capital on fixed-interest bank-loans, and the tradition of life-long employment which makes staff costs inflexible, imply that plants must be made to produce as great a volume of goods as can be managed. The practice seems to be to regard the cost of export sales as related somehow to the marginal cost of production. If exports are subsidized, the Japanese customer and not MITI meets the cost. The only long-term remedy is to sell imported goods competitively on the Japanese domestic market.

What *Nature* said eleven years ago

In November 1972, when a previous survey of science in Japan appeared (*Nature* 240, 185; 1972), the "oil shock" was still a year off and Mr Kakuei Tanaka's newly-elected government was concerned to know what use to make of the unprecedented growth of the previous two decades. Mr Yasuhiro Nakasone, Minister of State at the Science and Technology Agency, outlined his plans for the future to a *Nature* reporter visiting Tokyo and also kindly contributed a statement of his intention to the issue of 24 November.

Mr Nakasone is now the Prime Minister of Japan, so that his views of a decade ago have some special interest, if only historical. (And the Japanese courts are expected to issue a verdict early next month in the legal case that forced Mr Tanaka's resignation, turning on the accusation that he had accepted a bribe in connection with the sale of Lockheed aircraft to Japan Airlines.)

With concern about pollution of the environment virtually at its peak, it is natural that Mr Nakasone should have pointed to the need to match the application of the physical sciences with the development of understanding in biology. "Restoring the balance" is the phrase he used. He promised to devote much of his energy to the sponsorship of biology and the "soft" sciences. He emphasized the importance of the development of infrastructure (transport and so on) in Japan and pointed to one administrative embar-

assment of his post — the danger that the minister at the agency, committed as he must be to securing the budgets for atomic energy and space development, had little time for the general balance of the budget. These two major programmes are, however, still firmly under the agency's wing.

Otherwise, the 1972 issue of *Nature* contained several echoes of student troubles at the universities; the physics department of the University of Tokyo was occupied for much of the year. There was a gloomy opinion (from Dr A. Sibatani, a Japanese emigrant to Australia) about the prospects that the universities could be reformed and an admiring account by Dr Gilbert Devey of the National Science Foundation in Washington, DC, of a \$100 million project sponsored by MITI and the electronics industry, and coordinated by the Electrotechnical Laboratory, to design computers endowed with artificial intelligence. That is one MITI project that seems not to have fulfilled its sponsors' hopes; it has survived as a conspicuous component of the fifth generation computer project.

To *Nature* in 1972 it seemed that the most glaring fault in Japan's organization of science and technology was the government's neglect of university research, which was said to be "shamefully and needlessly" starved of resources. Readers may be able to judge for themselves how much has changed. □

These are the myths and half-myths about the success of Japanese technology. The reality is different. Japan's post-war successes have come in several distinct waves. How many Japanese now remember their pre-war dependence on the export of silk? Between 1950 and 1970, the most obvious component of technological innovation was modest (but not necessarily unspectacular) improvement of other people's technologies. If larger ships are likely to be cheaper (per tonne) to build and operate, why not work out the technology of building ships in the million-tonne range? If a large part of the cost of making steel is that of paying for the smelting plant, why not arrange that a furnace can operate more quickly?

But from the beginning, there were qualitative innovations — by 1960, for example, the Japanese optical industry had won a much-envied reputation for the high quality of its lens design — and found that its cameras would sell on quality, not just on price. And even now, when the steel industry everywhere is in the doldrums, Japanese companies are among the chief suppliers of high-quality spun-steel pipes as used in the oil and gas industries, a handy hedge against fluctuations in the cost of oil imports. Motor vehicles with two wheels have made their way in the world by being

technically more advanced than their competitors but Japanese cars, at first competitors for established industries on the grounds of cost (or productivity), have more recently begun to command attention for being cleverly designed. And it was Mazda, the Japanese manufacturer, that in the 1960s took up the Wankel rotary engine after manufacturers in the United States and Europe had declined to take the risk.

The more recent growth of the electronics industry, quantitatively spectacular, does not yet amount to a technological revolution (but may yet trigger off a social revolution). Four ingredients seem to matter — superb design, accurately anticipating users' needs, efficient and imaginative production engineering and careful quality control (so that products sold are usually found to function). The trick is to make the possible happen.

Why should these tasks be apparently easier in Japan than elsewhere? The only persuasive explanation involves people (including technologists) but not technology as such. First, as the pages that follow illustrate, those in charge of the direction of development appear to have recognized that junior colleagues probably have the most to contribute to innovation. Second, the general enthusiasm for improvement is infectious. Third, there is a widespread

The economy — science and welfare the losers

THE Japanese economy is not a textbook abstraction: you can see it. Suburbs packed with factories, small shops and restaurants in every niche, the clunk of aluminium money changing hands. The average time spent waiting for a taxi is less in Tokyo than anywhere else in the world: supply has grown to overwhelm demand. And to show that the customer is king, cab doors open automatically, and many drivers wear white cotton gloves.

One plausible explanation for the past two decades is that Japan is the only industrial state whose consumers have preserved peasant attitudes to shopping. What use is a cheap plough if it may break before the spring is over? Why buy a television set with 500 lines to the screen if sets with twice as many lines are on the market? The "boom", as the growth of the recent past is called, rests on the pocket-books of an army of discriminating shoppers.

In textbook terms, the result has been unprecedented. In 1982, Japan's gross national product (GNP) was ¥251,000,000 million — in round numbers \$10¹². (For currency conversion, the simple rule is that ¥1,000 is \$4, or just over £2.50.) In *per capita* terms, Japan ranks in national income only after the United States, Canada and the richer countries of Western Europe — Sweden, West Germany and so on. But what transfixes the curiosity of other industrialized countries is the speed with which this prosperity has been attained.

Japan took more than half a decade after

the ending of the Pacific war in 1946 (a year after the end of the war in Europe) to catch up with the pre-war economy. That done, the pace of economic growth was sustained at 9–10 per cent a year until the oil shock (a Japanese term) of 1973. 1974 was a bad year — output actually fell and inflation soared to more than 20 per cent in two successive years. But then the economy began to grow again, slowly at first but then more quickly — although not as quickly as in the 1960s. The oil shock has been permanent. People know now what they had forgotten — that an economy dependent on imports for essential raw materials, energy in particular, is not master of its own fate.

Three features of Japan's economy bear on the development of science and industry.

● The high savings ratio. On the average, the Japanese elect to save 21 per cent of their after-tax income each year, compared with an estimated 5.7 per cent in the United States. The incentive seems to be ordinary people's wish to save against their old age, especially important with the decline of the extended family and of the convention that each generation will shoulder responsibility for its predecessor. Moreover, seeking security, savers prefer to lend their money to the banks, at fixed interest rates low compared with those elsewhere. So the banks have plenty to lend on to industrial corporations even after helping to bail out the central government (see below). Accumulated personal savings are more than

a year's GNP

● Export-import trade. Japan is not undermining the industrial West by a cut-throat export policy. In many years, exports and imports, 16–18 per cent of GNP, are roughly in balance. What causes offence, however, is that Japanese companies, spurred on by built-in competitiveness and the burden of fixed-interest debt to the banks, sell manufactured goods to Western Europe and North America — and that the proceeds are then used for buying raw materials. The dollars (between 15,000 and 20,000 million of them) likely to be earned this year in trade with the United States will finish up in the hands of the oil-exporting states.

● Government debt. The government of Japan is in hock to its taxpayers. Taxes cover only 70 per cent of public expenditure, with the result that the government must borrow the remaining 30 per cent, much of it by forcing the banks to buy bonds at uncommercially low rates of interest. The causes include the political unpopularity of putting up taxes and the cost of anachronisms such as the commitment to buy surplus rice from farmers at above the retail price. Refinancing this mountain of debt may be difficult, in the years ahead, as the growing population of the retired starts spending its savings. So the government is strapped for cash, and must finance even essential projects on a shoestring. Science (especially in the universities) is one loser. Welfare is another. □

sense of personal responsibility, in research laboratories and on production lines, for seeing that allotted tasks are done successfully. And corporately, Japan now has a wealth of experience to show that the most sure route to riches from technical improvement is to be best. To be first helps, but is not essential.

This is where the economic climate of Japan is crucial. New projects mounted with such ambitious aims are bound to be expensive. The investment of the companies seeking to make a mark in biotechnology in what is, for practical purposes, a self-training programme (see page 372) is a vivid example of the scale on which resources are being invested in innovation. Financial stability and a high savings ratio obviously simplify the process. While there are now signs of the emergence in Japan of the kind of venture capitalism that tends to back innovation in the United States, for some time to come the willingness of large strong companies to branch out in new directions will be essential for the continuing change of pattern in Japanese industry.

In many ways, this has been the most striking feature of the "boom". While the growth of the economy has been steady, at least with the exception of 1974, the pattern of production has kept changing. Seiko, the company best known for its quartz wat-

ches, seems now to have recognized that it cannot continue to make them as cheaply as similar watches are made in Hong Kong, and is seeking other technologies to exploit, scientific instruments for example. From now on, as in the past decade, Japanese industry may be having to rebuild its productive base at intervals of a decade or so. Here again, it will have to accomplish this with the life savings of the Japanese people.

No wonder that Japan as a whole is asking whether the task will be as easy now that Japan is in the forefront of technology. Opinions differ, but it is striking that even in the fields in which Japan is strongest, electronics for example, there are surprising gaps in what Japanese corporations in aggregate have to offer. At first sight, for example, it would have been expected that Japan would be strong in the development of software for computing machinery. Teams of loyal programmers developing the software for a digital switching system for the telephone network would seem to fit ideally with the Japanese way of working. Yet NTT, the telephone company, says that the software may have to be imported (see page 364) to operate its new two-way broad-band communications system. More probably, suppliers such as Hitachi are right when they insist that the gap will

soon be bridged; the mystery is that NTT should have left this crucial task so late.

Much the same is true of Japan's computer industry proper. Japan's three leaders in the field, Fujitsu, Hitachi and NEC (short for Nippon Electric Company) have in the past 10 to 15 years been quick to follow the path of development in the United States. They and the other members of MITI's fifth-generation computer consortium (Mitsubishi, Toshiba and Oki) now have on offer a range of computing machinery comparable technically with what is available in the United States. Moreover, the first three manufacturers have judged it worthwhile to develop large capacity machines comparable with the US Cray and Cyber (Control Data) mainframe computers, capable of some hundreds of millions of arithmetical operations a second and used chiefly in defence command and control systems and applications such as weather forecasting.

What seems so far to be lacking in Japan's computer industry is the exuberance to be found in other sectors of the electronics industry when manufacturers sense that they have some substantial market in the palms of their hands. The usual explanation is that hardware has outstripped software, but the underlying difficulty may be more general — that in the

field in which the technology is changing quickly, and in which the ultimate users of computers are rarely able to specify with clarity just what they want to buy, manufacturers are not being helped to define their strategy by the loud and confident response of the market they have come to expect in other fields.

None of this, however, justifies the present wave of introspection in Japan about the underlying creativity of Japanese technology. Both in industry and government, the proposition is put like this: Japan has hitherto been an imaginative imitator of technological developments elsewhere in the world, principally in the United States. (For a robust justification of the practice, see page 381.) And while everybody knows that climbing Everest for the first time is the hardest task, in which false starts and expensive mistakes are bound to crop up time and time again, what if Japanese technology is somehow deficient in creativity, unable to pick out on the horizon of the future the Everests worth tackling?

This line of argument has provoked a great flood of anxious nail-biting. Some blame the educational system for its regimentation of its students. Some gloomily wonder why only four Nobel prizes have been awarded to Japanese scientists since the beginning of the century, asking whether that may not be a sign that Japanese society may be deficient in imagination. To outsiders, this whole train of thought is a self-indulgent irrelevance, based on a view of technology that is, in any case, false. People talk as if there is somewhere a hidden map of the technological future, and as if the question has now become that of knowing which parts of it will be coloured in by Japanese companies and their research people.

The mistake is to forget that technology is not an end in itself but (in high-sounding language) a means of satisfying social needs or (more down-to-earth) a servant of the market. The self-indulgence centres on the supposition that Japanese technology is already comprehensively competitive with that elsewhere (which is plainly not the case, for example, in aircraft manufacture or in the whole field of medicine). And the introspection about Japan's supposedly poor showing in the tally of Nobel prizes is an irrelevance because it should be clear to everybody that the most immediate explanation (if one is required) is the relative neglect of basic science both by the government and, more significantly, by students embarking on higher education, who still march predominantly into engineering.

Whether the years ahead will see this imbalance between academic science and engineering corrected is far from clear. It is, however, plain that nothing much will be accomplished without a radical change in the relationship between the government and the universities, necessary on other grounds and now long overdue. In deeply conservative Japan, the prospect that there will be such a change is far from bright.

Looking ahead to self-sufficiency

THE direct influence on Japanese science and technology is the minister in charge of the Science and Technology Agency, now Mr Takaaki Yasuda. Like his predecessor eleven years ago (see page 356), the minister sees his role in broad terms — helping to foster industrial development while providing the kind of technology that people need.

In the immediate future, the minister considers, there is a possibility that electronics will cease to be the engine of growth — “the end of the lode” may soon be in view. At the same time, the cost of technical development has grown to tax the resources of the government, and there is a need somehow to encourage the people concerned to be more flexible.

Japan's lack of natural resources remains one of the guiding principles — the chief justification of the nuclear energy programme (dependent on the agency for its direction and funds) but also the minister's conviction that the development of new materials and of biotechnology — “we must catch up” — are essential goals for the next few years.

Another guiding principle, more novel, is the need for international collaboration. Mr Yasuda refers to the several ways in which Japan has recently joined with other countries on scientific and technical projects, in oceanography, even robotics (as agreed at the Williamsburg summit) and other fields.

No doubt such developments will help to take the sting out of the continuing row about Japan's performance as a trading partner. But Mr Yasuda insists that the need for international collaboration springs from Japan's recognition that it cannot do everything itself.

On the space programme, however, Mr Yasuda says that Japan's interest in fields such as telecommunications implies that it needs to be a satellite builder, in which case reliance on other people's launching systems would make Japan “vulnerable”. Even so, Japan is looking into the possible use of the US shuttle for launching satellites. Nobody at the National Space Development Agency (see page 374) can yet guess which way the decision on the large rocket will go. While Mr Yasuda agrees that Japan's success so far owes much to the adaptation of technologies developed elsewhere, the going will be harder from now on. He advocates fuller collaboration between government, industry and the universities.

And where will it all lead? Well, a measure of self-sufficiency is an obvious goal. Beyond that, Mr Yasuda says that his agency should set out to find what people want from technology and to adjust the pattern of their activities accordingly. The task may be harder than Mr Yasuda suggests, given the general air of contentment with most of what has happened in the past three decades. □

This does not necessarily imply that technology as such will be held back, at least in the short run, but simply that the hankering after a richer intellectual setting in which Japanese technology can be embedded will remain unsatisfied.

Another Japanese tradition — the notion that a young person joining a company should expect to stay with it for the rest of his life — is probably another brake on the capacity of Japanese technology to throw up new projects. As things are, the successful companies are fully conscious of the need for flexibility, and claim to be able to redirect the objectives of their technical people without much difficulty. Obviously it helps that those concerned feel themselves to be part of a team whose success will determine their own personal prosperity. But there must be limits to what personal dedication can accomplish. There is a strong tradition in Japanese technology of recruiting senior research people (directors of laboratories for example) from government laboratories from which people are usually required to retire at 55 (and may leave earlier), but the relative immobility earlier on must be a drag.

Meanwhile, the ingredients of past success in technology will persist. The widely advertised robots now working away in Japanese manufacturing plants

will no doubt multiply and prosper, but the wonders of the past three decades derive from the personal qualities of the army of engineers that created the boom. Their technical flair, their willingness that it should be harnessed to agreed objectives within the group or corporation in which they work, their zeal for work and their enthusiasm for success are the true foundations of Japan's technology.

So the best guess is that the torrent of success (marred from time to time by glaring mistakes) will continue into the future (but see page 382 for some hints of what might go wrong). Those elsewhere who nurse the conceit that it should be possible to outsmart their Japanese competitors by launching on the world ideas that are somehow cleverer overlook the detail that has gone into what Japan has done. Japanese steel companies dominate the market for steel pipes because Japanese metallurgists and engineers have worked out how to roll pipes of high quality, consistently and on a large scale. The same is true in consumer electronics. Chagrined competitors have no choice but to follow the Japanese model but more thoroughly. And that, not the balance of payments, is the most striking measure of success: the boom is a model of how to do things that others must emulate if they, too, are to succeed. □

Parliament of Science

The end of the road?

THE Science Council of Japan (JSC), Japan's unique parliament of science, is in serious trouble.

On paper at least the ideal representative body of scientists and much envied elsewhere in the past three decades, the governing body of the science council is directly elected by all practising scientists (from universities, government and industry). The council has permanent access to the highest science policy body, the Council for Science and Technology, whose chairman is the Prime Minister and on which regularly sit the heads of all science-related ministries and agencies. What has gone wrong?

JSC has in the past three years fallen into disrepute with both the government and the technical community and has lost the greater part of its influence over science policy. It will be either abolished or totally reorganized in the months ahead. If there is a moral to the tale of JSC's decline, it is that grass roots democracy can only work when the majority of the electorate is prepared to put in the great deal of time and trouble it takes to make it work.

JSC was set up in 1948, in the aftermath of a sickening war and was intended as a fundamental reform meant to make more democratic the institutions of science and technology. In the 15 years that followed, before the "Great Boom", the council was led by Japan's leading scientists and fought hard for many popular causes.

One of its early objectives was that there should be properly equipped research institutes. Among the successes of this period

were the founding of the Institutes of Fundamental Physics, Solid State Physics, Space and Aeronautical Science and Protein Research. The council also had a powerful influence on the grant policies of the Ministry of Education. Inevitably, the fields in which individual members of the council worked were seen to be favoured. In the politest possible way, the council became in part a pork-barrel — an incentive for serious researchers to take it seriously.

At the same time, and with the then support of its members, the council took an independent political line, issuing a stream of declarations and recommendations to the government.

The dangers of the misuse of technology have been a recurring theme. In 1954 for example, the council passed a resolution, later incorporated into the Basic Act on Atomic Energy, that research on nuclear weapons should be prohibited and that research on nuclear energy should be guided only by the three principles of "autonomy, democracy and public accountability".

In 1962, fearing government interference in the universities, the council urged on the government a basic "Act on Scientific Research" including a statement guaranteeing the freedom of scientific research and thought. But this time the advice was less well received. The government declined to pass the act both then and when the proposal was revived in 1976.

The council has also gone its own way in international affairs, and was instrumental

in drawing attention to the radioactive contamination caused by the US atomic tests on Bikini Atoll in 1954. It called for the prohibition of the use of defoliants during the Vietnam war at a time when the government was committed to assist the United States by the provision of bases in Japan but was otherwise studiously neutral. The council began scientific exchanges with China, the Soviet Union, North Korea and North Vietnam long before Japan had opened diplomatic relations with them.

By the end of the 1960s, however, things had started to go wrong. Perhaps the decisive step was the decision of the Ministry of Education to set up its own special council to give advice on how research grants should be awarded. This move greatly reduced the power of JSC for it had no funds of its own to give but had made itself felt through influence over the ministry. At the same time, the government, preferring scientific advice free from politics, chose to rely more and more on the advice of the then expanding Science and Technology Agency. The government seems to have been particularly irritated by the encouragement of public opposition to nuclear power by members who appeared to speak on behalf of JSC and arranged that the council should have little influence on the expanding network of atomic energy institutes, controlled directly by the Science and Technology Agency.

The council has been on the skids ever since that time. As its influence with the Ministry of Education on research grants waned, university researchers found it more profitable to deal directly with the ministry than to channel their requests through JSC. And as the council's power declined, productive researchers lost interest in serving as representatives on the council. They were replaced by people — many of them with left-wing views — who regarded the council more as a political forum for launching criticisms of the government.

"The real culprits for the decline are Japan's scientists themselves", says Professor Ryogo Kubo, a physicist from the University of Tokyo, one of the few distinguished scientists who have been involved in the past year's last-minute attempts to reform the council. He complains that serious researchers simply lost interest in the council. "Sometimes whole faculties decided they would not bother to take part in council elections, even though JSC still had a major role to play."

Events came to a head in 1982. The government had by then decided that it had had enough of paying the bills for an organization that had become unnecessary in directing the course of science policy and was politically a minor irritant. A committee set up by the ruling Liberal Democratic Party concluded that the council's time as a government-supported organization was at an end. The government offered a stark choice. Either the council could become a

Science budget: facts and figures

AFTER two decades of phenomenal growth Japan is now spending more than six million million yen each year on scientific research and development — almost exactly a tenth of the world total. Japan's spending as a percentage of gross national product has recently drawn level with that of other industrialized nations (Japan 2.2 per cent, United States 2.3 per cent, United Kingdom 2.2 per cent); but the way research funds are generated and spent remains unique.

In Japan, a far higher percentage of total research funds is provided by industry (Japan 65.9 per cent, US 43.8 per cent, UK 40.8 per cent) and a far lower percentage by government (Japan 27.7 per cent, US 51.1 per cent, UK 51.7 per cent) than in other nations. In part, this huge difference can be accounted for by the very high level of expenditure on defence research in the West (around 15 per cent of total research funds), most of it necessarily added to the government research bill, relative to the tiny amount spent in Japan (0.7 per cent).

But even taking into account all funds spent on defence the government of Japan still contributes significantly less to total scientific research expenditure than other countries (Japan 25.4 per cent, US 33.2 per cent, UK 31.6 per cent).

One consequence is that as industry provides virtually all its own research funds it is particularly concerned with making research produce marketable products quickly and efficiently. As a result there is an overall bias towards applied and product orientated research — with benefits which have been obvious — rather than basic research.

But now the Japanese government is worrying that too great a fraction of the nation's research funds is going into applied research. With a view to the long-term future it is trying to increase basic research spending in industry, government institutes and the universities — the difficulty is finding the money with the government already in trouble over its high level of borrowing.

private organization with a limited amount of government support, or it could continue to exist with its democratic elections replaced by a system in which representatives would be appointed by the government.

Since 1982, the council has been in turmoil. In the best traditions, it confessed the error of its ways — principally of having lost touch with its own electorate — and in November 1982 came back with a compromise proposal by which two-thirds of its representatives would be elected and one-third nominated by the government. This was promptly rejected by the government, which nevertheless countered with another compromise, partly from a wish to arrive at a quick solution amid the confusion caused by the resignation of Prime Minister Zenko Suzuki.

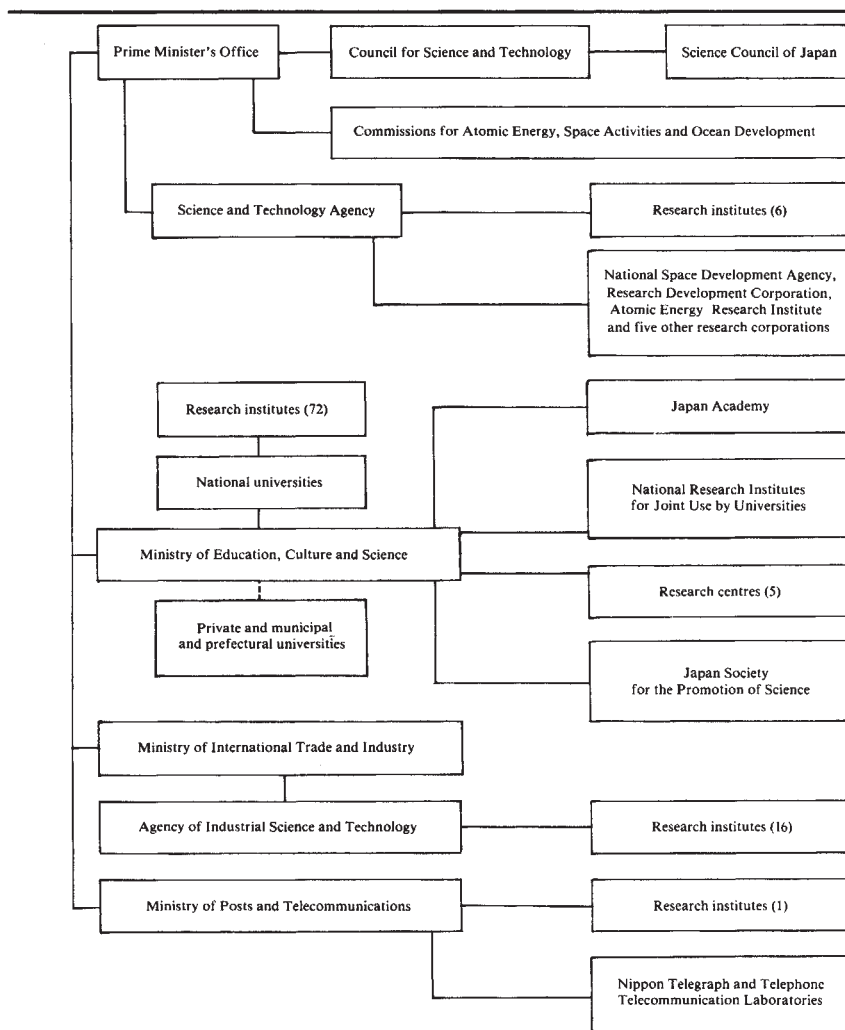
The idea was that the JSC representatives would neither be appointed by the government nor elected by the scientific community but instead would be appointed by the larger scientific societies. Professor Kubo was at this stage elected chairman of the council and has spent the past year trying to find some way of making the government's compromise workable.

Kubo has become a specialist in the arcane niceties of electoral systems. His chief problem was to find some system to accommodate the many different societies of different size and with different constitutions. The system which he claims was "the best we could hope for within that framework" fell back on the old device of an electoral college. Scientific societies would at their own expense appoint (or arrange for the election of) representatives who would then go on to elect the final representative council (with the government meeting the cost).

The government rejoiced and began to implement the Kubo formula by pushing a bill through the upper house of the Diet. But then, in June this year, the General Council of JSC decided it could not agree to the formula. Professor Kubo felt he had no choice but to resign.

There are now three groups left within JSC; those who supported Kubo's compromise, those who favour fighting on in the hope of wringing a better deal from the government and a large group of diehards who believe even abolition is better than compromise: better no science council at all than one that is not truly totally independent of the government.

So now the Science Council of Japan is in a state of suspended animation. The election of representatives held every three years and due this month has been postponed to give the Diet, just returning from its summer recess, time to consider its next move. Negotiations with the government continue. Politically, responsibility for finding a compromise acceptable to the government rests squarely with the council, which as at present constituted seems better able to say no than to devise constructive compromises. □



Running science — a bureaucracy explained

JAPAN'S top policy maker is the *Council for Science and Technology*, a ten-member advisory committee chaired by the Prime Minister and including the Ministers of Finance and Science and Technology and the president of the *Science Council of Japan* (see page 361). Besides setting general policy the council can also support research directly through "special coordinating funds".

Other commissions within the Prime Minister's office take special responsibility for atomic energy, nuclear safety, space activities and ocean development.

Fourteen ministries and agencies have scientific research budgets; the big four are shown in the chart.

The *Science and Technology Agency* (annual budget $Y320 \times 10^9$) acts as a secretariat for the various councils and commissions and runs "big" science — nuclear energy, space and ocean development — through a series of research corporations.

The *Ministry of Education, Culture and Science* ($Y60 \times 10^9$) funds basic scientific research at three research institutes, six National Institutes for Joint Use by Universities, and the 93 national universities.

MITI, the *Ministry of International Trade and Industry* ($Y73 \times 10^9$) supports scientific research with industrial application through its research institutes attached to the *Agency of Industrial Science and Technology* and through contracts with industry. Among the major national projects under way are: fifth generation computers, new energy development (the Sunshine project), energy conservation (the Moonlight project), and next generation industrial technology (see page 373). MITI also helps set up and fund cooperative research by industrial companies which later compete in marketing the final product. The very large scale integrated circuit project (1976–80), often mistakenly thought to have given Japan the world lead in 64K random-access memory production, was one such project.

The *Ministry of Posts and Telecommunications* has nominal control over *Nippon Telegraph and Telephone* the public telecommunications monopoly which has three laboratories, equivalent to the US Bell Laboratories but a quarter of their size. It was research here that gave Japan the world lead in optical fibre production technology. □

The electronic society

Commuters go on-line

CAN the world's major supplier of electronic hardware also pave the way towards the electronic society of the future? The next few years will show which way the wind is blowing.

Indeed, this autumn will see a start made on a colossal 15-year telecommunications project designed to make Japan the world's first all-singing all-dancing "advanced electronic society". The long-term aim of the Information Network System (INS) is nothing less than to unify the country's separate telephone, data communications, telex, telegraph and facsimile transmission systems into one single two-way optic fibre network carrying voice, data and video traffic.

The idea is simple enough. Given commodious optical fibres, techniques for digitalizing analog signals and the recognition of the economies of digitalization, it makes sense that there should be only one channel of communication to (and from) each user of electronic signals. But as things are — and like most other places — Japan has a hotchpotch of different kinds of channels for different kinds of services, while the public monopoly in charge, the Nippon Telegraph and Telephone Company (NTT), has been slow off the mark in digitalizing its telephone switching system. So why not leapfrog ahead, and digitalize everything in one go?

The model system being built for service later this year will allow use by about 10,000 selected subscribers in the Musashino-Mitaka area of Tokyo, the suburb which is the site of NTT's research laboratory, in every way the equivalent of AT&T's Bell Laboratories in the United States. The chosen few, probably still counted as lucky by their neighbours, are to try out INS terminal equipment free of charge over the next five years.

The most exotic of all the new services will be provided by a broadband optical fibre network capable of carrying video and high-speed facsimile signals. These services are aimed at providing all the benefits of face-to-face conversation between users miles apart. Groups of people will be able to see and speak to one another over two-way video links as well as to "hand over" documents through a facsimile transmitter that can send an A4 page in two seconds. There is also a device called a video sketchphone, which has the advantage of operating with a lesser bandwidth (carrying 64 kbits a second) that will transmit drawings and doodles written on a special pad to a video screen so that they can be used to accompany a telephone conversation.

The facsimile transmission network is to be expanded and digitalized along with the telephone service so that a whole range of centralized computer-controlled services

will become possible. Facsimiles can be centrally stored and then forwarded later (if the recipient's line is busy for example) as can telephone messages, which will be stored in a centralized form of telephone-answering machine. Digital telephones and exchanges will also be able to supply customers with some more mundane services, immediately giving the calling number and call charges and allowing links of more than two telephones for multi-participant conversations (conferencing is the word).

Particularly important in the model system is the emphasis being given to teletext. The network will be linked to the public access data bank CAPTAIN, just coming into service with about 20,000 pages of general service information (weather forecasts, train timetables and the like). In its digitalized version, CAPTAIN will be used in the model system to provide voice as well as graphics and, through links to appropriate computers, will offer a rich menu of services such as home banking, theatre and hotel reservations and tele-shopping, as well as mere information. With such services, NTT believes it will make the biggest impact on ordinary people's lives.

When the Tokyo model network is complete, NTT will embark on a demonstration system for the Tsukuba Expo-85 Science Exhibition designed to show off the system to the 20 million visitors from Japan and overseas expected to attend. Then the plan is to offer the full range of services, even to rural areas, by the end of the century and thus begin the "advanced electronic age".

Integration and digitalization of the different telecommunication services has already begun; the national telex and digital data networks will absorb the telex network in 1984, the telegraph network in 1985 and the facsimile network by about 1990. Will humble Musashino-Mitaka be the cradle of a new civilization?

NTT officials talk as if it must be. They are naturally excited by the immensity of the project and by the prospect of being the first in the world to integrate computers and telecommunications in an operating network, not an exhibition demonstration. But there is a risk, NTT director Ei Shikiba says, that "that time we'll be first in the world and will have no precedents to follow. We'll have to be ready to encounter failures."

But could the whole idea be a failure? Outside NTT, it is easier to find critics than supporters of INS. Electronic equipment manufacturers say that the provision of centralized store-and-forward services is totally unnecessary. If people want an answerphone, they can buy one from a shop. If they want equipment that stores a telex message until a line comes free, they

can buy that too. So why, with INS, make people pay for services they may not need? (Lucky Musashino-Mitaka subscribers may enjoy immunity from extra charges, but NTT will have to pay for the national revolution it is planning through its computerized billing service.)

Computer users are also sceptical. They say that integrating all data transmission services into a common network is unnecessary, given that the next generation of microcomputers will have the power of today's mainframes — only a few local networks are needed for special applications. Those who have witnessed the resounding lack of success of teletext elsewhere in the world (for example, Prestel in Britain) are gloomy prophets, saying that even it and related services such as teleshopping will be lead balloons. The sceptics may, however, have overlooked one crucial factor in the Japanese market: the apparently inexhaustible wish to buy a better version of one's neighbour's latest acquisition.

Two special groups of critics have special axes to grind. Small shopkeepers who with the aid of MITI have kept supermarkets out of Japan see teleshopping as another threat to their businesses and (unlike video-capable terminals) have a constitutional right to vote. And high school students, already spending 2-3 hours of their evenings at *juku* (cramming schools), fear that teachers will invade even their homes once two-way video systems are in service. The threat is not far-fetched: some *juku* are already using telex for that purpose.

Other critics of INS take a more knowing line. Thus Professor Hiroshi Inose of the University of Tokyo, by trade a signal digitalizer, says that much of the fuss about the "inauguration of the advanced information society" by means of INS is nothing but a smokescreen. NTT will have to raise enormous sums simply to update and digitalize the telephone system. Inose's own surveys have shown that the electronic service people most of all want is home security — the ability to telephone their homes to check that burglars have not yet broken in and that the gas and electricity are properly switched off. Such facilities are already being built as standard by some apartment construction companies and they do not require a new digital network, says Inose.

NTT director Shikiba, however, is not prepared to give an inch to the critics. He shares the view of the originator of the INS concept, Yasusada Kitahara, that integration of telecommunications and computers is the inevitable next stage in the evolution of human communications.

To those who are worried about the social effects of INS, Shikiba stresses that the whole point of building a model system is to find out what people do and do not want and that NTT is taking enormous trouble to carry out surveys and to listen to local people. Already discussions

Science news in fashion

THE technology boom has been followed by a boomlet — a proliferation of popular science magazines which jostle with the soft-porn and comic books in even the smallest corner bookstores in Tokyo. At least seven science magazines are normally found on the shelves together with a great number of personal computer magazines and two or three more scholarly scientific journals. This boom is very recent. Not one of the six best-selling indigenous magazines, now with total sales of three-quarters of a million copies a month, had appeared before 1981.

The popular science boom is spearheaded by *Newton* which, with a circulation close to 400,000, sells four times as many copies as its nearest competitor. *Newton* was launched after careful study of the US *National Geographic Magazine* and of *Geo*, and can now fairly lay claim to being the world's best illustrated science magazine.

Newton's interests span a wide spectrum of science: space launches, particle physics, plate tectonics, galactic evolution, and solar eclipses are all explained in staggering multicoloured features alongside articles on the latest in high technology — lasers, tokamaks, microchips, electron microscopes — and interviews with Japan's leading scientists. Most of it, perhaps reflecting editor-in-chief Professor Takeuchi's physics background, is "hard" science: what is not there is "pop" psychology or the kind of quasi-science that is supposed to be the stuff of best-selling magazines.

Among *Newton's* competitors are a couple of look-alikes, apparently based on the policy that "the more we look like *Newton*, the better we'll sell". Direct imitation of parts of *Newton* graphics is common. But in the business climate of Japan, in which



savage competition is applauded and legal action frowned upon, copyright lawyers are not much engaged. Nobody seems to know what has provoked this sudden boom in popular science magazines. Is there a new and serious interest in science among young people? Or has science finally become sexy, as is suggested by the appearance of the Nobeyama radio observatory chief Professor Norio Kaifu and the story of his millimetre wave telescope — "The Big One" — in an advertisement for vitamin A?

Supporting evidence? When *Nature* published "Dramatic growth of mice that develop from eggs microinjected with metallothionein-growth hormone fusion genes" last year, it became a full front-page

lead story in the evening editions of all four of the main newspapers (total circulation 80 million).

Newton's associate editor Kazuo Terakado admits to being frankly puzzled. He does not see a deeper appreciation of science emerging in Japan, rather that nobody had thought of publishing a magazine like *Newton* before. He may be over-modest. The man on the Yokohama subway car has nursed throughout the "great boom" a fierce pride in Japanese technology. As the establishment's ambition to make a bigger mark in basic science is consolidated, what could be more natural than that ordinary people should want to learn the map of their next conquest? □

are taking place in the Mitaka community to find out how the system can best serve it. To makers of stand-alone equipment, he says that NTT has no wish to cut them out. "Competition is best — if they can provide cheaper services, let them do so."

The development of software that people will really want to use is more difficult. The participation of potential users, especially in the specification of teletext services, is hoped for. If necessary, "the aggressive introduction of US software" will go ahead.

In the long run, perhaps the most important feature of the INS model system is one so far unsung. The system will involve not only one small suburb but also a link to one of Tokyo's main business areas, an area where a great many of the Mitaka residents work. A few companies will be offered the opportunity to experiment with a satellite office in the suburbs linked

to the main office by videoconference and high-speed facsimile transmission services. The obvious advantage is that workers would not have to spend two or three hours each day on an overcrowded train. The snags? The death of the social institution of the after-hours drinking session in the subway-station corridor. Catastrophic unemployment among the railway workers who cram extra passengers into overcrowded coaches. A serious threat to the principle that women's work — the care of the family — cannot be shared by their wage-slave husbands for lack of opportunity.

Even so, it may be that the real future of INS will lie in this direction. Video transmission and other services requiring high bandwidth cable will not become widely available unless there is a staggering reduction in the cost of optical fibre or a breakthrough in video compres-

sion techniques. Sony's director of research, Makoto Kikuchi, sees the idea of INS as something of an *ooburoshiki* (literally, a giant wrapping cloth) — a larger than life project which is more of a boast than a real aim, but the kind of boast that propels people forward to a very respectable achievement. Did not the US space programme in the 1960s yield that great practical innovation the non-stick frying-pan?

Many critics of INS's more grandiose statements thus consider that it will end up offering its services to the chain of major Japanese cities and a huge number of satellite offices surrounding them. The average home will benefit from little more than the digitalization of the telephone system and perhaps the occasional use of teletext. But even if only that much is achieved, it will be a massive gain for Japan's exhausted commuters. □

Universities

An elite well established

FOR ambitious young people, and the children of ambitious parents, a decent university education remains the eye of the needle through which a person must pass on the way to personal success. So, if the government will not or cannot provide a place in higher education, you must be prepared to pay for it.

These propositions explain two of the most striking features of Japan's educational system — the intense competition at the high-school level for places at a university, and the coexistence of two parallel systems of universities — those supported directly by the government and called national universities, of which there are 97, and those whose constitution is that of a private non-profit corporation. Altogether, Japan has 446 universities, including 34 public universities supported by prefectural governments as testaments of duty to their local taxpayers diligently discharged.

Tokyo alone has no fewer than 100 universities of different kinds, including at one end of the spectrum powerful national universities such as the University of Tokyo and private universities such as Waseda, and at the other institutions specializing narrowly, say in literature, and with fewer than 1,000 students.

The competition for university places among the high-school population, familiar everywhere, has been carried to extremes in Japan. It is common for students to prolong their high-school days with a few hours of private tuition in the evening, at least in the year or so before they are due to take the tests of attainment and aptitude that qualify for entry to the national

universities. Success brings some relief, for although the standard requirement for a four-year undergraduate degree is that a student should have picked up 72 recognizably different credits, examination standards are not so high.

People worry about the consequences of intense competition for the curriculum, at the high-school level. Well they might. There is another more palpable but insidious consequence of the competition for university places — it continually reinforces the reputations of the prestige universities as the places to which students should seek entry.

In Japan, people are willing seriously to discuss questions such as whether Kyoto (number 2 on most scorecards) is gaining on Tokyo. (Kyoto has its four Nobel prizewinners as a counterpoise.) The discussions are not entirely meaningless, for they can fall back on data derived from the high-school scores of their new entrants. Naturally, the universities take the top slice from the pool of candidates available, thus reinforcing the positive feedback between reputation and quality. So the high-school competition is not simply to win a place at a university. Which university is what matters, and a student's placing on the examination lists will help to determine his career.

Out and out elitism of this kind is not, of course, unknown elsewhere (although West Germany has cleverly managed to avoid its worst excesses). It might not be as serious in Japan if it were not that the ever-present influence of the Ministry of Education in the affairs of individual universities, requiring uniformity of academic practice,

discourages the hope that lesser universities might be able to improve their standing.

None of this seems to have deterred young people from following their own or their parents' wishes, and thronging into the universities. Indeed, higher education is the most conspicuous social investment engendered by the "boom". Between 1960 and 1975, the numbers of students in higher education multiplied by more than three (to 2.2 million), counting the students in Japan's junior colleges (post-high-school institutions concerned with teacher training, technical education and the like).

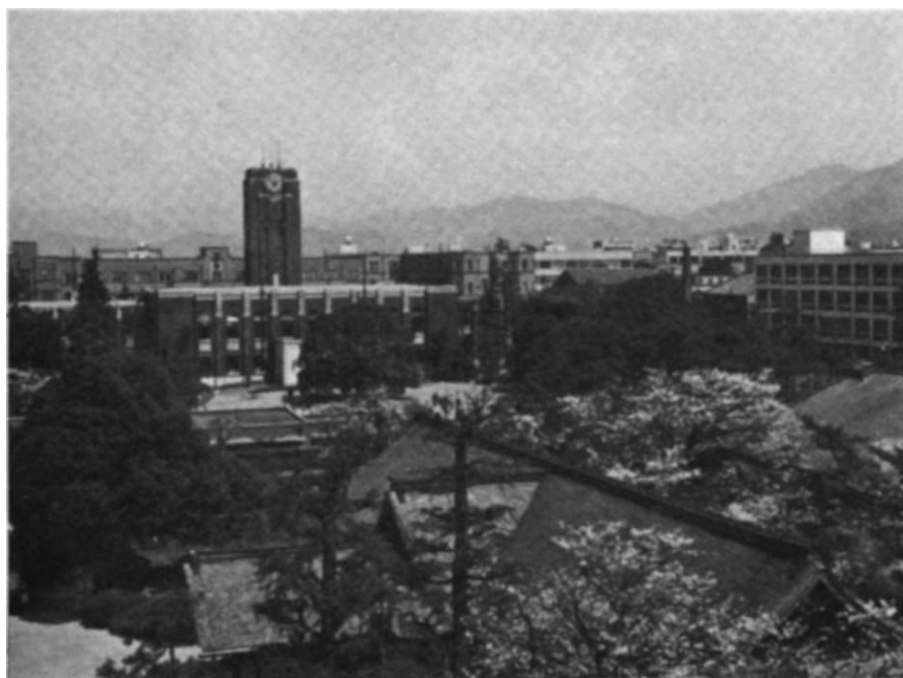
Most of this spectacular growth has been in the private universities. The national universities have not quite doubled their intake of students since 1960, while the private universities have absorbed the extra demand for places and now take five times as many entering students as the national universities.

The curriculum is rigidly determined both by the accreditation procedures operated by the Ministry of Education (applying to private as well as national universities) and by the direct and detailed control exercised by the ministry on the national universities and, by extension, on the private universities which necessarily emulate them. The first two years of the standard four-year undergraduate course are for general education (including foreign languages), but students must usually declare their long-term interests at the outset. There follow two years of specialist education towards the end of which, at the research universities, undergraduates may find themselves working as research assistants while keeping up with their instructional timetables.

The position of women in Japan is nicely illustrated by the proportions of women entering different kinds of institutions. In 1981, 22.1 per cent of university entrants but 89.6 per cent of junior college entrants were women. Technical schools (which provide training for people already at work) included only 2.2 per cent of women in their intake for the same year.

Students' preferences for different courses of study are a consistent proof of the Japanese interest in engineering. Of the 1.73 million undergraduates enrolled at Japanese universities in 1981, no fewer than 334,000 were following an engineering course, exactly six times as many as students in the natural sciences (including mathematics). There were in 1981 more students of agriculture than of science proper, many of them in the specialized agricultural universities scattered throughout Japan. Many universities have no science faculty, only engineering.

Not even at the national universities is education free, although there is a scholarship scheme that makes it virtually so for a small proportion of students. The official calculation is that the average cost of education at a national university works out at just under Y300,000 a year. (The averaging process takes account of the low



Kyoto — number two to Tokyo

A case of neglect

WHATEVER the praise showered on the electronics industry, Japanese academics appear to harbour a sense of being unfairly neglected. That at least was the tenor of a long conversation at the University of Kyoto some weeks ago.

Geography is obviously important; Japan's nearest neighbours are China and South and North Korea, but Japan's science looks at least across the wide Pacific to the United States. So the university, conscious of the importance that its academics should stay in touch, reckons that as many as 1,000 of its faculty will travel abroad each year, mostly for visits of a week or a few weeks. But it seems to be agreed that this is insufficient — and no substitute for a healthy flow of scientists from abroad, people who would stay for a little longer than the usual whistle-stop tour crammed in after some international conference has ended.

There has always, of course, been a steady trickle of long-stay visitors from elsewhere whose academic interests (history, language or business, perhaps) have given them a professional interest in Japan, but scientists have been few. Kyoto, however, has been first among Japanese universities to take advantage of a recent change in the government's regulations for the universities allowing the appointment of foreign nationals to university staffs in spite of their status as civil servants, and will on 1 October be welcoming Professor G. Hall, from the University of Nottingham in the United Kingdom, as a professor of chemistry. Other universities are keen to follow suit. The ministry in Tokyo is eager to stress the importance of this change, and indeed insists that it is doing everything it can within the confines of the budget to encourage foreign travel in and out. But the budget is tight.

Seeing other people's faces is not the whole of what academics want, however. The Japanese community is asking why it should be that even when it has done all the right things (built accelerators, millimetre wave telescopes and the like), worked harder than people elsewhere (laboratories are routinely occupied late into the evening, while graduate students often work all night) and published in English, its work is so often neglected.

The temptation is to reply, perhaps facetiously, that everybody feels his work is neglected, or to point to the evidence from citation studies that people refer most readily to the work of people known to them personally. Certainly all those concerned with the management of scientific journals know how frequent are complaints that an author has deliberately overlooked another's work. There is, however, in Japan some compelling chapter and verse to sustain the charge that the unacknowledged use of Japanese ideas happens too often. □

living costs of students at home.) Tuition fees at private universities are three times as great in science subjects, while the cost of a medical education may be ten times as great at a private rather than a national university.

At the public universities, national and prefectural, academics are public servants and are paid accordingly, on published salary scales. Japanese-style increments are built in. Salaries range from ¥100,000 a month at the bottom of the heap to ¥500,000 at the top, supplemented with payment of a bonus three times a year worth five months' salary, and are poor compared with what people with scientific reputations can hope to earn in industry. Even with allowances for having dependents or for having to commute to work, the monetary rewards of teaching at a public university cannot account for the people's eagerness to be academics, but social status is worth a great deal. And opportunities crop up for supplementing incomes by means of consultancy with industrial companies.

Private universities are a different case, enjoying as they must the mixed benefits of financial autonomy. Government subventions have been increasing since the 1960s but not so rapidly, as the government explains, as to compromise their independence. The result is that the pressures of market forces are directly felt even at the level of the department.

The central administration may keep for its own purposes a proportion of the tuition fees accruing to a department, which will then be free to use the remainder for educational purposes and research. So departments unsuccessful in attracting students are likely to find themselves penalized by means of a high ratio of students to staff, and with little left over to help with the cost of research.

Even so, it is curious that in the past year the government has decided to phase out the elements in public grants to private universities spent on teachers' salaries. Even universities such as Waseda, sustained not merely by its high reputation and the generosity of its alumni, fears the consequences of the increased fees that new entrants will in future be charged.

The financing of research in the national universities probably represents the most labour-intensive method ever devised for distributing small sums of money. Funds trickle from the Ministry of Education under two headings — bread and butter support calculated on a complicated formula in whose design equitability has triumphed over need, and project research grants costing (this financial year) close on ¥40,000 million on whose distribution under twelve distinct headings no fewer than 1000 academic reviewers and a small army of officials will adjudicate.

Formula support is an attempt to acknowledge that research is a part of an academic's work. In the traditional national universities, the appointment of a

professor to a chair entails that the government should also provide three further posts, two assistant professors and one research assistant or vice versa. The boss is then given to spend on research a share of the national cake (this year, ¥90,000 million) calculated from the nature of the department (experimental or otherwise) and its teaching responsibilities (graduate students or not).

Such teaching units (called *Koza*) may well under this heading be allotted up to ¥7 million — and will then find that half of it or more is kept back by the central university to cover administration costs. Many teaching units find themselves left with ¥2 million or so, enough to cover the cost of routine equipment but not much else.

The ministry's ordinary research grants have much the same status in successful academics' research planning. One obvious deficiency is that they cannot be used as a means of recruiting short-term assistance. How could they in a society in which life-long employment is the norm? And why, in any case, do graduate students exist? The result is that people compete energetically for the ministry's smaller grants (and help with publication costs is often essential) as if to neglect to do so would be regarded as an acknowledgement of failure. But a grant by itself will normally be insufficient.

Within this pattern, however, the ministry does hand out some substantial grants. It will build generously on internationally acknowledged success: Japan's next Nobel prizewinner need have no anxieties about further research support. Since 1982, six grants have also been awarded under a new scheme allowing three or four year support for projects costing in total two or three hundred million yen (in round numbers, \$1 million). One of these grants, for example, supports Professor Masamichi Tsuboi's well-known molecular biology at the University of Tokyo.

Demand for this kind of support has been enthusiastic — and is largely unrequited. The ministry has also a scheme for supporting special research projects, usually involving collaboration between groups in several universities (resembling some West German practice) prepared to work together in an important area of fundamental research, while there is an imaginative scheme for supporting university-industry collaboration (each side paying its own costs).

The obvious defects of this pattern of support for basic research is that too large a proportion (nearly two-thirds) of the inadequate fund available is distributed without regard to the quality of what is being done, while much of the remainder (more than a half) is distributed in penny packets, by a mechanism made clumsy by the bureaucracy of ostentatious equitability which succeeds in giving academics the sense that the ministry, not they or their university, is the arbiter of what research

should be undertaken.

So people turn with relief to industry. Money does not necessarily change hands directly between a principal investigator and his industrial partners, but equipment can and does, while academic scientists have access to machine shops and other facilities not normally found in universities in Japan. And the companies involved in these collaborations usually also recompense the department indirectly, by making a "donation" to the university.

The snag here is that graduate students will find themselves almost exclusively occupied as high-grade technicians on projects of a short term character — how to fit a television camera into a catheter, or how best to build some novel instrument or machine. An established professor, of course, can take a broader view, but there appear to be many cases in which his assistants work primarily as research managers, keeping graduate students up to the mark and teaching them what they need to know. Those who insist that Japan must find ways of being more creative may have to look no further than this. Links with industry go deeper than this. Often, for example, the chemistry department at the University of Tokyo provides bench-space for people from industrial companies with a common interest in the problems being tackled.

Some of the consequences of collaboration are physically visible. For it stands to reason that a group of companies that has enjoyed a fruitful collaboration with some group of academics should couple its next donation to the university with a suggestion that the department could with advantage be better housed. The other side of this coin is also apparent. The department of biochemistry in the Medical School at the University of Tokyo is, for example, housed in a building so little like a modern laboratory (it is the building put up in a hurry after the university was virtually destroyed by fire after the 1923 earthquake) that some of its occupants seem to have lost heart. When will the biotechnology industry change all that?

But where are the post-doctoral fellows in this pattern of academic research? For practical purposes, they do not exist. The convention and practice is that after the five-year PhD stint is completed (and there is a break-point at the two year master's degree), a person will either step into an academic post or take a job in industry. The notion that a career in science should include two to four years when a qualified person might concentrate exclusively on research is foreign to Japan — so many Japanese scientists take themselves off to the United States and elsewhere to enjoy a little of this distinctive blend of intellectual luxury and material hardship.

For better or worse, the ministry seems to have recognized that something may have to be done to create a holding pattern for PhD scientists. Mr K. Kawano, head of the research grant division at the ministry,

Tsukuba bucks the system

THE new University of Tsukuba is Japan's first ever attempt at radical university reform. Planned in the early 1970s, after student rioting had highlighted the stagnation of Japanese universities, Tsukuba is a success for the reformers — but not yet so great a success that Tokyo and Kyoto have been shaken into asking whether they too need reform.

To escape the rigidity of the faculty and departmental system, Tsukuba has simply done away with it. Instead, research and teaching are quite separately run. The 2,000 members of staff carry out their research at the university's 26 research institutes and 18 research centres, which have some of the best equipment in Japan.

The 14,000 students belong to colleges grouped into three broad subject clusters — fundamental sciences, cultural and biological sciences, and engineering and management — plus schools of medicine, health and art. The curriculum is set upon American lines — it is up to the student to choose between courses and to collect the necessary credits.

Also gone is the chair system, which allows its incumbent to control the funds assigned to it. Professor Fujiwara (Institute of Information Science and Electronics) says that giving each member of staff his own funds has had "a very good effect on the standard of research".

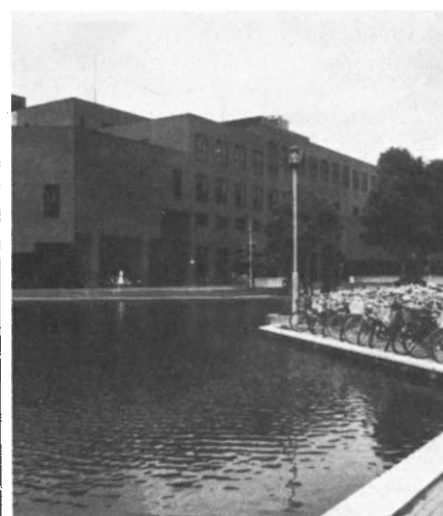
Flexibility in the research system is encouraged by including both interdisciplinary projects and joint projects with government and industry. Professor Fujiwara's own institute is, for example, compiling a chemistry data base with support from the Science and Technology Agency, working on a computer-aided decision and design system for MITI and producing a science and technology encyclopaedia for a non-profit foundation.

Tsukuba has also had a crack at doing away with the pre-entry examination hell. A quarter of the university's places are left open for students selected by high-school recommendation or interview alone rather than by entrance examination, and there has been no apparent decline in the quality of the entrants.

Tsukuba certainly looks and feels different from a normal national university. Out among fields and forest in the northern part of Tsukuba science city, it has plenty

of space — a bicycle is essential — and plenty of bright new buildings decoratively arranged around quadrangles and ponds. It even has plenty of tennis courts, a big plus. (The pressure on flat land in Tokyo is such that people travel for an hour and a half for a game of tennis.)

But there is another characteristic of the national universities also missing from Tsukuba. There are no massive sign boards set up at the gates exhorting students, in giant *kanji*, to join this or that political faction. And there are no left-wing students, red-helmeted or masked, bellowing political messages through high-powered



Tsukuba — a tight ship

megaphones each lunch-time. Student freedom at Tsukuba, it is gradually revealed, is not "American style". Although every university has rules and regulations, Tsukuba is one of the few that actually apply them. One student said darkly, "You have to learn the difference between Tsukuba's face to the world and the reality". Student meetings cannot be held without staff approval. And one of Tsukuba's outspoken critics was recently prevented from going to Tsukuba to give a lecture. It would be wrong, however, to think of Tsukuba's students as seething with discontent under iron repression. Many of the staff are surprised (and no doubt pleased) to find their students are quiet and diligent. What is the explanation? "Well, it's a beautiful environment here, and you can always play tennis." □

says that it is hoped to have a set of recommendations early in 1984, with the intention that the ministry's science council (not to be confused with the Science Council of Japan) will then be able to make a recommendation to the politicians. Given the general worry about the budget, it will be a surprise if the outcome is dramatic.

The outlook for a more radical change in the university system is not bright. The suspicion that other ways of operating universities might be beneficial has led to

the creation of universities organized on a different pattern, as at Tsukuba. But there is not general agreement that change is necessary. And it goes without saying, in Japan, that nothing substantial could be done without the agreement of such a complicated network of committees and consultative bodies that the casting vote will remain with the powerful establishment of Tokyo and Kyoto graduates who ask what can be wrong with a system that has done so well by them. □

National Research Institutes

Building a new breed

THE myth that basic and innovative research in Japan lags behind that of the West is rapidly being destroyed by the three new Ministry of Education institutes at Okazaki, for Molecular Science, Basic Biology and Physiological Science. But the superb facilities and the international atmosphere at Okazaki are not typical of Japan's universities. Rather, they result from the creation of a new open style of institute, the National Research Institutes for Joint Use by Universities (NRIJU), specifically designed to provide researchers throughout Japan with the opportunity to do world-standard work.

What makes NRIJU unique is that each institute has independent status as a national university. This neatly overcomes the problems that had troubled earlier "national" institutes; either they were attached to a university and came to be regarded as that university's personal territory, or they were attached to a ministry, where they were independent enough but could not confer the professorial status which would attract the very best staff. The NRIJU formula is already proving a massive success. Several thousand visitors a year are visiting Okazaki to run their experiments on top class equipment, and whole areas of experimental science have been rescued from neglect.

The oldest and largest of the three institutes, the Institute of Molecular Science, is now in its seventh year but building continues. The latest project will be the world's first synchrotron radiation source designed specifically for molecular chemistry, providing an intense source of ultraviolet radiation. The 600-MeV injector is now being tested and parts of the 53-m circumference storage ring are lined up in the semi-underground main hall, all ready to be joined together.

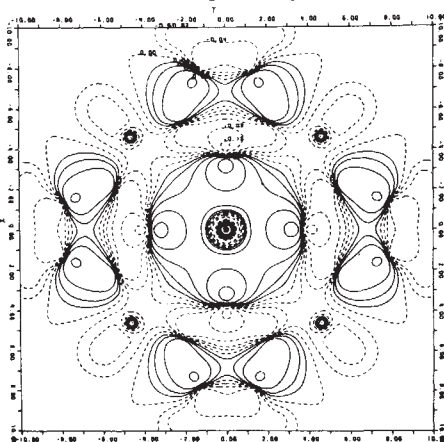
Later this year, eight ports will provide synchrotron radiation from 10 Å through to the infrared for optical absorption and emission spectroscopy, photoelectron spectroscopy, photochemistry and studies of molecular dynamics. And the flat roof of the main hall has proved ideal for tennis courts — not, apparently, because researchers have time to play tennis but because the courts reassure local people that whatever goes on underneath will not blow up the whole neighbourhood.

The synchrotron is expected to attract several thousand more research visitors to the institute each year. Already the institute may have thirty visitors in a day. Why so many? "We have the best quality and the most up-to-date equipment in the world," said Dr Iwao Yamazaki of the Instrument Centre (qualifying this modestly with "Well, that's 80 per cent true"). What this means, Professor Hiroo

Inokuchi explained, is that the institute's 100 or so staff have "two jobs, their own research and serving visitors from other universities". And as in every Ministry of Education facility, there are far too few technicians.

The overall effect has been to improve the level of research in Japan. The new computer centre, for example, with a remarkable fully-automated unmanned control system, now carries out one tenth of the world's molecular orbital calculations. Before the centre was built, Dr Hiroshi Kashiwagi explained, "only two or three people in Japan were calculating molecular orbitals. Now there are more than 100."

But what happens to the visitors when they go back to their own ill-equipped laboratories? Apparently they start applying for Ministry of Education grants, a welcome development given that one



A molecular orbital map produced at the Institute of Molecular Science's new computer centre.

indicator of the sad state of university research is that only a third of those qualified to apply for grants-in-aid ever do so.

The high standard of facilities at Okazaki also means that the institutes are putting themselves on the international map. As the director of the Institute of Physiological Sciences, Professor Koji Uchizono, put it, "Now, with the best facilities in Japan, we've finally reached the level where bilateral exchanges are possible"; foreigners can be invited from the West without fear of embarrassment. Indeed, more than 120 foreigners made the trip to the Institute of Molecular Sciences last year, and from the outset have served as institute councillors, one of the first being Professor Sir George Porter from the Royal Institution in London.

Where will this lead? What everyone would like to see next is the expansion of the new institute system. Next on the list is an Inorganic Complexes Institute, to be built either at Tsukuba or Okazaki. □

Fundamental physics

Japan is an island

KYOTO University's Yukawa Hall, housing the Research Institute for Fundamental Physics, is unique in having been set up with the express aim of being "open to the world". But despite many successes, the distance separating Japan from other advanced nations and the problems of gaining fluency in a foreign language still present barriers to the kind of free exchange of researchers that is taken for granted in the United States and Europe.

The institute was the brainchild of Hideki Yukawa who won Japan's first Nobel prize in 1948 with his prediction of the existence of the pi-meson. Returning from a spell at Columbia University in the United States he had the idea of setting up an institute modelled on the Princeton Advanced Studies Institute — one that would contain a small core of permanent researchers, be managed by all the country's universities, and have its facilities open to researchers both within and without Japan. At the time when the institute was set up in 1952 these ideas were very new and the institute's eventual budget for academic exchange exceptional; now the National Research Institutes for Joint Use by Universities share many of the same aims.

The institute has grown to include a total of fifteen permanent researchers and a unique one-year post allocated to a foreign visitor. But there are problems in getting really top scientists from abroad to fill the post. Leaders in the field feel that Japan is just too isolated and that one year is too long to be away from the main debating grounds of theoretical physics, even in this electronic age. "Plenty of people would like to come for a month" explained Professor Humitaka Sato, a former director of the Institute, "but not for a full year". Those who do come are still going to find it difficult to have regular contact with more than a limited group of people. Although more and more people are mastering foreign languages, Kyoto still hasn't reached the sort of polyglot levels found in the University of Zurich where, when a foreigner is present, seminars begin with a decision on whether the proceedings should be conducted in English, French or German! Young researchers in Japan find it very hard going to express their ideas in English — despite an astonishing reading ability — and a visitor is not going to be able "to just drop in on students for a casual chat".

The difficulties in bringing foreigners over to Japan are partly made up for by growing opportunities for Japanese to go abroad. Conference trips to the United States and Europe, difficult to come by a few years ago, are now commonplace for

professors even though much battling with red tape is required for each trip. Most professors can also spend a couple of years studying abroad early in their careers. There's opportunity for exchange of ideas too at the institute's "Kyoto Summer Schools" which are rapidly gaining a high reputation and attracting more than thirty foreigners each year.

Despite the difficulties of really being a part of the international community the institute has many achievements to be proud of. Professor Yukawa who remained head of the Institute until 1970, was successful in stimulating exchange of theoretical physicists within Japan and actively promoted areas of science, like astrophysics, which were new to the

country. His own rejection of gauge theories in favour of "fundamental domain theory" meant that he took a radical, and unproductive line far from the mainstream of theoretical physics. Those who were directly influenced by him found their ideas badly received in the wider world. But theoreticians who followed their own bent did much better: Professor Sakata's composite model of hadrons, completed in Murray Gell-Mann's Nobel prize winning work in 1969, Professor Hayashi's models of star formation which won him the Royal Astronomical Society medal in 1970 and Professor Sato's own work on relativistic astrophysics are all big successes helped along by this small institute. □

Planning for the future

Looking for creativity

CLOSE to ¥80,000 million is being spent this year on two government projects specifically aimed at stimulating the basic and innovative research at which Japan has for so long been thought weak. Of the two projects, MITI's Research and Development of Basic Technologies for Future Industries and the Research Development Corporation's project Erato, the second is truly revolutionary. Erato (Exploratory Research for Advanced Technology) sets out quite deliberately to smash the restrictive pattern of research organization found in the universities, government and industry.

Willingness to admit the need for change in the research system has come, says Erato

The Erato projects

Ultra-fine particles: The exploration of the properties of ultra-fine particles which find application in magnetic memories and superconducting materials.

Amorphous and laminar materials: Design and synthesis of amorphous metals, laminar compounds, and non-equilibrium compounds to create new materials, such as high-corrosion resistant materials and transparent magnetic materials.

Fine polymers: Synthesis of plastics to which special functions are imparted, such as biocompatibility, semiconductivity and superconductivity.

Perfect crystal: The potential of defect-free crystals in developing a new group of semiconductor devices which use the electrostatic induction effect.

Bioholonics: This takes its inspiration from a book by Hiroshi Shimizu expressing views similar to those of Arthur Koestler's *Janus*. The main project is development of a biological machine — an actin-myosin powered motor.

research director Genya Chiba, only because "in the late 1970s, we finally gained sufficient confidence in our own technological abilities to admit we had been copying Western technology for the previous hundred years. Now we've reached the frontiers of technology, we've

lost our teachers in the West and have to try our own innovative research."

Chiba identifies numerous obstacles to creative research in Japan. Most laboratories tend to be closed and inward looking and allow little interaction between people of very different backgrounds. Their hierarchical organization allows little freedom for researchers and prevents younger scientists from developing their own ideas. "The way younger people are stifled by their superiors is a serious problem", says Chiba, "although it's not so bad in industry, where older people can always be shifted into management." In industry though, research is almost entirely product-orientated, giving little scope for real innovation.

Erato would sweep away these obstacles and usher in a new age of youth, freedom and diversity. Only young people are allowed to join the teams of twenty or so researchers — with the single exception of the project director, the age limit is 35. Researchers are deliberately chosen from diverse disciplines and backgrounds — approximately equal numbers from government research institutes, universities and industry — and special attempts are made to recruit foreigners, the first time ever in a government research programme.

Research is carried out in independent rented laboratories deliberately spread across the country, where there can be complete freedom from the restrictions of already established institutes. And perhaps most important, the Research Development Corporation guarantees funds for five years but does not set a specific goal for the project. Chiba likens his role to that of a film producer. "We put up the money, set the general theme and pick the director. It's up to the director where things go from there." Chiba hopes that with this much freedom, a "new, native Japanese science may emerge", or something very un-Japanese may occur — "we may find one crazy maniac in this system who has really

new ideas".

Five projects are now under way (see table) and a sixth on bio-information transfer is about to start. All are original and one — on bioholonics — quite eccentric; perhaps mirroring the project's name Erato. Chiba says she is the "Greek goddess of arts and science" but *Brewer's Dictionary of Phrase and Fable* defines her more narrowly as "the muse of erotic poetry". Those who are confused can always use the project's other code name, MEDAS, for Marvel Exploration by Dynamic Aggregation System — whatever that might mean.

The first project to get under way, ultra-fine particles, has now been running for two years. It is too soon to say whether it will succeed, but Chiba seems pleased that it has used the time to "explore all sorts of different directions and only this year will start to concentrate on particular areas". The aim is to keep launching new projects every year until about ten are running.

The MITI project seems quite humdrum by comparison. Cutting any talk of the philosophy of creativity, MITI's Agency of Industrial Science and Technology simply sees that Japan does not have a firm technological base in some areas which are firm bets as growth industries during the coming decade — new materials, biotechnology and new electronic devices (see table). Research in these areas takes too long, is too expensive and is too risky for private companies, so MITI will pay for and help do the research instead.

MITI officials openly admit that Japan is behind the West in many of the new project's areas, but do not claim a magic formula to help catch up — just the right facilities and hard work.

To speed progress, though, research is being carried out "in parallel"; in the

MITI research and development project of basic technology for future industries:

New materials: High-performance ceramics, synthetic membranes for new separation technology, synthetic metals, high structural plastics, alloys with controlled crystalline structure, and advanced composite materials.

Biotechnology: Bioreactors, large-scale cell cultivation and recombinant DNA.

New electronic devices: Superlattice devices, three-dimensional integrated circuits, fortified integrated circuits for extreme conditions.

effort to develop new high-performance ceramics that can operate at extreme temperatures, for example, research is being carried out simultaneously on both silicon carbide and silicon nitride materials.

The project has now been running for almost two years and MITI is well pleased with progress. There had been fears that because of its long-term nature, the project would have been one of the first to be cut now that the recession is biting. But MITI officials are not disposed to throw away the chances of long-term gains for a small saving now, and the project is to be kept going at the planned rate. □

National space programme

Time for decision

JAPAN'S policy on the commercial exploitation of space is in a cleft stick of the government's own making.

For the past fifteen years, it has carried through a programme of development aimed at acquiring all the ingredients of a full-blown space programme — launching facilities, tracking stations, rocket manufacturers and satellite builders. But the system that has been developed is too small to satisfy the needs of the principal users of satellite services, the telephone company NTT in particular. So should Japan swallow its pride and launch most of its satellites on other people's launchers, or should it build a bigger rocket and stomach the cost and the delay that would be entailed?

The problem has its roots in the 1960s and the decision by the deliberative body called the National Space Activities Commission that scientific satellites should be left with the University of Tokyo, but that there should be a new self-contained organization to look after practical applications. The National Space Development Agency (NASDA) was set up in 1969. Its terms of reference preclude military activities, but government policy, reaffirmed in 1978, requires that it should develop the techniques needed for a "comprehensive space programme", with the proviso that "it is not necessary to produce everything domestically".

Since its beginning, the agency has been a kind of impresario for the space programme. Its rocket technology is not home-grown but depends on the construction (under licence from McDonnell-Douglas in the United States) of the rocket engine originally built for the Thor intermediate-range missile. Part of NASDA's objective has been to foster familiarity with rocket technology in Japanese industry, whence the emergence of Mitsubishi and Nissan as rocket manufacturers and Toshiba and Mitsubishi as satellite constructors. NASDA maintains a launching complex (called a space centre) on Kyushu (the southernmost island of Japan) and a design and development institute at Tsukuba, but otherwise is commendably lean, with fewer than 1,000 employees. NASDA will this year be spending some 80 per cent of the total space budget of ¥113,000 million. (The Space Science Institute will rub along on 8 per cent of the total.) NASDA will also recover some ¥20,000 million from users such as NTT.

The problem of the rocket motors derives from the agreement with the United States under which the Thor engine design may be used in Japan, and which prohibits military uses (no problem), transfers to third parties (again no problem) but which also singles out this engine as the most

advanced that could not have military applications. The chances of negotiating a better deal seem small.

NASDA's strategy in the past decade has been to make the most of the basic engine design, using up to nine strap-on boosters to increase the initial lifting capacity, and seeking better performance for the two upper stages, developed in Japan. The immediate result is NASDA's N-11 system, capable of putting 350 kg into a geosynchronous orbit. And by using liquid-hydrogen fuel for the second stage NASDA reckons that it should be possible to increase the payload to 550 kg.

The obvious snag, made plain earlier this year by NTT, is that telecommunications

satellites need to be twice as massive. So NASDA, which has planned tests of the cryogenic rocket system (called H-1) in the next three years, is already brooding about what should happen afterwards.

The options are to develop from scratch a larger first-stage rocket, perhaps by bolting together engines of the kind now used, to acquire the design of a simpler larger rocket from the United States or deliberately to let NTT and the broadcasting agencies have their satellites launched by other people's rockets.

With the prospect that the squeeze on public spending will be still further intensified in the months ahead, NASDA will be lucky if it emerges from the review now under way with its old ambitions intact. But it would be a surprise if the government were to go the whole hog and release NASDA from its commitment to build a comprehensive capability. □

Space research

Satellites on a budget

MOST governments with ambitions in space follow the obvious administrative course and set up an appropriately named space agency. For a time in the early 1970s, however, Japan made do with the University of Tokyo and a string-and-sealing-wax operation.

Japan's first satellite, the tiny 21-kg *Osumi*, was put into orbit on 11 February 1970 by the university's Institute of Space and Aeronautical Science (ISAS). Since then, the institute has launched an average of one scientific satellite a year in what must be the world's most economical space programme.

Unlike its bigger and younger brother, the National Space Development Agency (NASDA), the institute has developed all its own space rockets. It also runs its own independent rocket and balloon launching sites — all at an annual cost that even in 1982 was less than \$40 million. The secret is in part extremely simple. ISAS has built bigger and bigger rockets by repeatedly adding new and larger first stages to smaller rockets already launched successfully.

The first rocket launched, in 1955, was only 23-cm long and appropriately called "Pencil". The 30-cm "Baby" followed in 1956 and then the "Two-stage Baby", 150-cm long and capable of carrying a camera. In 1958 came the first of the Kappa family of rockets, Japan's distinctive staircase into orbit.

Modular design is the keynote. Kappa 6 weighed some 270 kg and reached an altitude of 60 km. Kappa 8, the next, was the first stage of Kappa 6 mounted on a new, more powerful first stage, and reached 200 km. In 1960, the first stage of Kappa 8 was combined with the whole of Kappa 6 to make the three-stage Kappa 9 that flew to 350 km. Then the institute built a new first-stage rocket to carry various

stages of the Kappa series to found the Lambda family. In 1970, Lambda 4S took Japan's first satellite into an elliptical orbit. The fact that Japan became the fourth country to launch a satellite is less striking than that the University of Tokyo is still the only academic institution to have done so.

This "do it yourself" approach has not always been easy. Even the biggest launchers were little more than glorified sounding rockets, so that they lacked stability and control. It actually took ISAS five attempts to put Japan's first satellite into orbit. And the satellite, alas, expired after 17 hours.

A series of successes quickly made up for these early difficulties. The more powerful and better guided Mu rockets put eight scientific satellites into orbit by the spring of this year and established Japan as the leader in X-ray astronomy. Now, ISAS has grown into a proper national institute as one of the National Research Institutes for Joint Use by Universities.

EXOS-C, equipped for infrared spectroscopy of the middle atmosphere, will be the next satellite up in the spring of 1984. But the really big step comes in 1985 with Japan's first deep space probe — a flyby mission to Halley's comet. Astro-C (1987) and CXGT (1990) will continue the tradition in X-ray astronomy and, in a cooperative project with the United States, ISAS will contribute one satellite to the NASA Origins of Plasma in the Earth's Neighbourhood (OPEN) programme.

NASDA is said to cast envious glances at ISAS's successes and to be keeping its smaller brother in place with a limit on the maximum size of rocket it is allowed to launch. ISAS officials, though, would only say diplomatically "Yes, we too have heard such rumours". □

Biotechnology

Missing the boat?

Is biotechnology the one that got away? This is the question provoked by the attempts now being made by pharmaceutical and chemical manufacturers in Japan to emulate what has been happening in the past five years in the United States. Non-scientists also take an interest: "We are second, don't you think?"

In retrospect, however, it is a little mystifying that Japan has been so slow off the mark in this potentially important field. In 1972, the then minister of science and technology Nakasone went to great lengths to emphasize the importance of the life sciences (see page 358) while in the same special issue of *Nature* attention was drawn to the importance of the then recent development of a technique for manufacturing L-lysine by fermentation (using molasses as a starting point). The following year, the Mitsubishi group of companies lent its support to the Nagasone proposition by setting up a new laboratory (the Mitsubishi-Kasei Institute for the Life Sciences) to spend Mitsubishi money on basic (and open) research in the field.

Yet now Dr Hirotohi Samejima, managing director of the Kyowa Hakko Kogyo company, the pharmaceutical arm of Kyowa Hakko, says that the development of biotechnology in Japan is "still in an early stage". What has been happening this past decade?

Kyowa Hakko is precisely the kind of company that might have been designed for the exploitation of genetic manipulation. Its technical and financial strength derives from a process for the production of alcohol by fermentation using gel-encapsulated yeast cells but it was also the company that developed L-lysine production by fermentation. (The product is being used in Japan as a supplement for cattle food, and a production plant is being built at Cape Girardeau, Missouri, in the United States to produce 7,500 tonnes of the material a year.)

Like many of its Japanese competitors, Kyowa Hakko is now two years into a programme of development in biotechnology. The company has been manufacturing beta-interferon on a 2,000-litre scale from genetically engineered *Escherichia coli*, and, in conjunction with the National Cancer Institute (Tokyo), a clinical trial is under way. Work has also begun on the production of gamma-interferon.

How has all this been accomplished? The company maintains a research laboratory with more than 200 professional people in a northern suburb of Tokyo, with the understanding that a quarter of them will be responsible for acquiring and using the skills of genetic manipulation to give the company an innovative place in the marketplace. But that will take time, which is why Kyowa Hakko has signed several

research licensing agreements with companies overseas, Genentech (US) and Jenssen (Belgium) for example.

The Genentech arrangement will give Kyowa Hakko the Japanese rights to market tissue plasminogen activator, for example. Among other things, the Tokyo laboratory is looking at the use of cell fusion techniques in case it should seem sensible to manufacture monoclonal antibodies and the diagnostic materials that might be fashioned from them. This project is "still at the research stage".

Kyowa Hakko is thus in a condition now all too familiar elsewhere in the world: it knows that biotechnology should have a place in its operations but has not yet been able to decide just what that will be.

Technically and administratively, however, the company is in a good position to exploit any procedure that emerges from its enquiries. The financial records suggest that the company can well afford to take a long view of the future, perhaps looking for a niche in the marketplace as much as a decade from now. The immediate lack seems to be that of a mechanism to suggest what the niche may be.

Does MITI help? Like the electronics companies, Kyowa Hakko is anxious to put the ministry in its place, insisting that MITI has neither the power nor the competence to shape the pattern in which new industries develop. But it also approves of some of the collaborative projects that the ministry has sponsored in the past two years — the search for serum-free media for the cultivation of cells, for example.

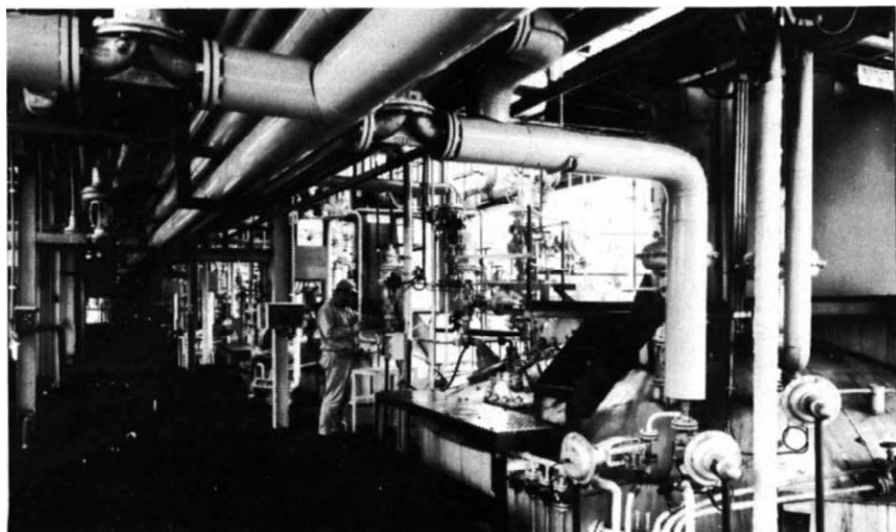
The Mitsubishi Chemical Company is in much the same state of development. Again, there is a research and licensing agreement with Genentech, and the company has an interest in materials such as plasminogen activator, serum albumin (for which there is a known market of the order

of 100 tonnes a year) and the endorphins. But Dr Wataru Yamaya, general manager of the chemical company's life sciences department, says that as things are there is a "lack of basic knowledge" which again suggests that development will take time. Mitsubishi, again, can afford to wait. It plans to take a close interest in the agricultural applications of biotechnology.

For the Mitsubishi group as a whole (which, technically, is forbidden to be a fully integrated industrial empire as before the Second World War), biotechnology makes sense because it would in principle allow a move from its traditional business (coal, coke, steel and heavy chemicals and engineering) to products of whose sales-price a large proportion is added during manufacture. Dr Yamaya says that such a strategy has been made logical by the increased cost of raw materials. How soon biotechnology will be able to contribute to it in significant ways is an open question.

Elsewhere, there seems to be an air of quiet frenzy about biotechnology. Collaboration is the order of the day. For example Suntory (best known for its whisky) is embarking, in partnership with Biogen, on a long-term project to produce the poorly characterized material called tumour necrosis factor, whose clinical application must be years away and even then doubtful. Government ministers and their agencies repeatedly emphasize the importance of biotechnology — and MITI has set up its network of committees to stimulate research aimed at the development of some important techniques. Naturally enough, academic researchers are taking a close interest.

The reasons why, with the high reputation of many Japanese groups in molecular biology, this is happening only now are far from clear. The most plausible is that collaboration between universities and industry functions well only in respect of short-term projects. But the sheer lack of numbers of academic scientists with an appropriate training in biology must also be a serious handicap. □



Amino acid fermentation at Kyowa Hakko Kogyo

Tsukuba science city

Museum or powerhouse?

UNLIKE its Siberian cousin, Novosibirsk, Japan's own science city at Tsukuba, 60 km north-east of Tokyo, has yet to win worldwide fame. But the monumental labour of building Tsukuba is now nearly finished. Twenty years of construction and more than a million million yen have created the biggest and best equipped science city in the world: 46 government research institutes, two universities and 11,000 research scientists — almost a half of all Japan's government scientists — have moved to what was originally poor farmland. Soon the city will grow further as thirty or so high-technology companies move in.

But Tsukuba was always intended to do more than just provide new and better laboratories; in the 1960s, its planners dreamed of creating a city of scientists with a whole new environment where innovative research would flourish as never before. Has Tsukuba fulfilled part of this dream? Or is it true, as a cynical official of the Science and Technology Agency said of Tsukuba's vast, and largely empty, acres, that "Tsukuba is not a city, it's a drive-in museum of top-class government facilities"?

Tsukuba's beginnings are, in any case, found more in practical necessity than in any kind of idealism. Twenty years ago, government research institutes were in a mess; they were overcrowded, out of date and some even dangerous. Simple rebuilding or expansion was often impossible. Many of the institutes were located in Tokyo where land prices had gone through the roof. A mass move to a new site seemed an obvious solution.

At the same time, the big cities had become appallingly overcrowded. The government was under pressure to take the lead and move some of the population — and some of the jobs — to new towns outside the big cities, and an example was provided by the success of Senri, a new town built outside Osaka to serve the world Expo 70 site.

There was also a more general desire to recognize the vastly increased importance of science and technology to the Japanese economy and to revitalize research. A new science city, its proponents thought, could provide a chance for reform; a new kind of university could be built that would give a wider education to undergraduates and more freedom to researchers and a whole new creative environment would emerge.

In its provision of new facilities, the success of Tsukuba is obvious. The list of the new institutes that have been built (see table) is matched in length only by the list of major new facilities they contain: the Building Research Institute's test floor, on which an entire eight-storey building can be constructed and shaken to pieces by simulated earthquakes, the National Institute

for Environmental Studies' large laser radar, NASDA's giant space simulation chamber, the Electrotechnical Laboratory's microfabrication clean rooms and synchrotron light source, the National Research Institute for Metals' 175,000-gauss superconducting magnet, the National Research Institute for Research in Inorganic Materials' high-voltage electron microscope with a resolving power of 2 Å, the National Chemical Laboratory's superconducting FT NMR spectrometer. . . .

Whether Tsukuba is or will be a stimulating environment is another matter. First of all, Tsukuba is vast — and mainly empty. "Central" Tsukuba is not so much a city as a strip of land 18 km long by 6 km wide, flanked by a pair of four-lane highways. Surrounding this strip is an area ten times as large — half the area of Tokyo — designated for suburban development but at present containing just a few small farming villages. In all this area, there are just 127,000 people. Tsukuba's landscape is one of isolated clumps of laboratories, or high-rise blocks of government housing among fields, while, far off in the distance, other towers, belonging to unknown institutes, vanish in the haze.

Second, Tsukuba is not proving particularly popular. A high percentage of scientists, particularly the more senior staff, prefer to commute from Tokyo than to move to Tsukuba. And this is despite the nightmarish journey involved; an hour's train journey takes you not to Tsukuba, which despite its "city" status has no station nor any connection to the country's freeway network, but to one of two small country towns 10 km away. Then comes a queue for a bus or a 3,000-yen taxi-ride.

The reasons why many people do not want to live in Tsukuba are obvious. It has almost no cultural or social facilities. What it needs is a massive injection of theatres, concert halls, cinemas, restaurants, department stores and all the good things of life to be found in Tokyo. And it needs superb senior high schools, for the highly-educated parents of Tsukuba will not risk sending their children to country schools when they have already learned how much in life depends upon success in the university entrance examinations. And perhaps Tsukuba also needs more of a centre of some kind.

Even so, more than a few of the new residents are enjoying a style of life where going off to work or visiting friends means jumping in a car "Los Angeles style" rather than taking a ride on a crowded subway. Worst off, of course, are housewives; staying at home in Tsukuba is duller than staying at home elsewhere.

New Facilities at Tsukuba Science City

Higher Education and Training Group

The University of Tsukuba
The University of Library and Information Science
National Laboratory for High Energy Physics
National Education Centre Annex
Tsukuba Botanical Gardens, National Science Museum
Tsukuba International Centre
Tsukuba International Agricultural Training Centre

Construction Group

National Research Centre for Disaster Prevention
Tsukuba Telecommunication Construction Engineering Development Centre (NTT)
The Geographical Survey Institute
Public Works Research Institute
Building Research Institute

Science and Engineering Group

The National Research Institute for Metals
National Institute for Research in Inorganic Materials
The Tsukuba Space Centre
The National Institute for Environmental Studies
Tsukuba Administration Office of Agency of Industrial Science and Technology
National Research Laboratory for Metrology
The Mechanical Engineering Laboratory
National Chemical Laboratory for Industry
Fermentation Research Institute
Research Institute for Polymers and Textiles
Geological Survey of Japan
The Electrotechnical Laboratory
The Industrial Products Research Institute
National Research Institute for Pollution and Resources
The Meteorological Research Institute
Aerological Observatory
Meteorological Instruments Plant,
Japan Meteorological Agency

Biological and Agricultural Group

Tsukuba Primate Centre for Medical Science
Tsukuba Medicinal Plant Research Station
Agricultural Research Centre
National Institute of Agricultural Sciences
National Institute of Animal Industry
Fruit Tree Research Station
National Research Institute of Agricultural Engineering
Sericultural Experiment Station
National Institute of Animal Health
National Food Research Institute
Institute for Plant Virus Research
Tropical Agriculture Research Centre
Forestry and Forest Products Research Institute
Tsukuba Seed Testing Laboratory

Common Facilities

The Tsukuba Centre for Institutes
The Japan Information Centre of Science and Technology (JICST), Tsukuba Branch

Private Research Institutes

Foundation for Advancement of International Science
Institute of Ocean Environmental Technology
Japan Foundation for Shipbuilding Advancement
The Japan Automobile Research Institute, Inc.
The Experimental Farm, Nippon Agricultural Research Institute
Centre for Better Living
Testing Laboratory
ZEN-NOH Central Feed and Livestock Institute



Tsukuba — termed an “academic new town”

Third, Tsukuba has not so far really broken down the barriers between different institutes and managed to encourage the kind of cross-disciplinary contacts that were promised. Efforts have been made. When the city was built, it was divided into sectors by putting close together institutes carrying out similar sorts of research, even when they belonged to different ministries. The “construction sector”, for example, contains the Science and Technology Agency’s National Research Centre for Disaster Prevention as well as the Ministry of Construction’s Geographical Survey Institute, Public Works research Institute and Building Research Institute. All take a strong interest in earthquake research, but from different angles. This was a fine idea, but there are signs that simply putting institutes close together is not enough. The Tsukuba Centre for Institutes tries hard; designed to be “the place” for contact and interchange among researchers, it supports research societies, publishes a newspaper, provides a club and even organizes tennis matches. But most researchers still live their lives without venturing from their own institutes. Indeed, most are not even sure what institutes might be found in the further reaches of Tsukuba.

Many at Tsukuba would acknowledge these problems, but given time, they say, the city will “fill in naturally” and develop its own character and culture. The oldest new residents have been there only ten years, hardly long enough for a new city to grow up. Others feel that the massive International Science Exposition planned for 1985 will put Tsukuba on the map and attract more of the people, and the high-technology companies, the city wants. Others again feel that massive spending on cultural facilities — the kind of things the government is notoriously loathe to provide money for — is still absolutely necessary. Only time will tell whether Tsukuba will be simply a collection of institutes, or whether it will usher in a golden age of innovative science. □

High-energy physics

Joining the big league

WITH project TRISTAN rest Japan’s hopes of taking the lead, at least briefly, in experimental high-energy physics. If the accelerator’s 3-km main storage ring is completed on time in 1986, then Japan should be able for two years to operate the world’s highest energy electron-positron collider — 30 GeV per beam — until it is in turn overtaken by the LEP 50 GeV per beam accelerator at Geneva in 1988.

Or so at least it seemed. But since the plans were laid, the competition has not been inactive. The collision energy planned for TRISTAN will, on paper at least, be outdone at the Stanford Linear Accelerator Center (SLAC), where the plan to build bending magnets beyond the exit of the linear beam promises energies of 50 GeV per beam by 1986 — ahead of both TRISTAN and LEP (see *Nature* 303, 562; 1983).

TRISTAN, however, has more to offer in the future. It has been designed so that a proton storage ring powered by superconducting magnets will run through the same tunnels as the electron ring, giving electron-proton collisions at $25e \times 300p$ GeV, perhaps by 1989. Plans for other electron-proton colliders — notably at DESY near Hamburg — are still at an early stage.

The building of the accelerator and indeed the founding of the institute that houses it — KEK — is a major triumph for Japan’s high-energy physics community, which has been struggling to obtain world-class facilities for more than 30 years. After the Second World War, research in nuclear science was prohibited by the Allied Occupation Forces together with research in rocketry and other areas that might have military applications. Not until the peace treaty of 1951 did discussion of building accelerators begin. By 1956, an Institute for Nuclear Structure had been built but its facilities — a 60-inch cyclotron built in 1955 and a 1 GeV electron synchrotron in 1961 — were tiny compared with the machines then appearing at Brookhaven in the United States and at the European Organization for Nuclear Research (CERN) in Geneva.

At the outset, there were many physicists bent on pushing ahead to build a high-energy proton synchrotron, but as Professor Tetsuji Nishikawa, director of KEK’s laboratory at Tsukuba, puts it, “we wasted almost a decade with discussions on the scale of the budget, the effects on research programmes in other fields of science as well as the organizational problems of establishing a new national laboratory”. The time went on “battles with other scientists, not the government”.

The new High-Energy Physics Laboratory, KEK, was authorized by the Diet in

1970 as the first of a new kind of research institute, known as the National Research Institutes for Joint Use by Universities (NRIJU). (see page 372.) A 12 GeV proton synchrotron was completed in 1976 and a “photon factory” (a synchrotron radiation source) last year.

Now KEK’s top priority is to get TRISTAN finished as quickly as possible and then, by some big success, to put KEK on the map. Already the 8 GeV accumulator ring which will take electrons from the photon factory’s 2.5 GeV linac is nearly complete and work on the main ring has begun. But there are signs that the Ministry of Education will not supply funds at the rate required, so that the project will be a year or two late. Nishikawa points out ruefully that even with the building of the new institute, Japan’s total funding for high-energy physics is still a much lower fraction of its gross national product than either Europe or the United States, and that “LEP and SLAC are not going to be slowed down”.



KEK director Tetsuji Nishikawa

TRISTAN project director Satoshi Ozaki is utterly determined that KEK will push ahead and run experiments before LEP is ready. “Even if the machine is half crippled, we must be running at the world’s highest energy by 1986”, he says. “After all, you can say that the cultural depth of a nation is proportional to the energy its accelerators have reached. To reach a high energy shows not only that the country has the will to do basic research but also the ability to develop the technology required.”

TRISTAN was originally designed to search for a range of new particles in-

cluding the top quark, heavy leptons, the Higgs particle and the Z^0 as well as for the study of quantum chromodynamic jets. One of the key prizes — the Z^0 — has already fallen to CERN, but that still leaves plenty to look for. And if rumours that CERN have found the top quark prove to be true then TRISTAN may be the ideal place to study its physics.

Looking into the future, research is in progress on the superconducting magnets that will power TRISTAN when it becomes an electron-proton collider. A proton accelerator — the only one in Japan — has already been built and has exceeded expectation by reaching a top energy of 12 GeV in its main ring. Two beams are being extracted: one is split to provide proton, pion, enriched kaon, antiproton and hyperon beams and the other is used

for bubble chamber experiments. As the synchrotron's injector can provide more protons than the main ring needs the excess is being put to use in a special facility for solid state research, and in experimental cancer therapy at a newly-established Particle Radiation Medical Centre of Tsukuba University. Eventually the proton synchrotron main ring will feed into the superconducting ring of the TRISTAN project.

Right now, the staff of KEK is keeping its fingers crossed that in the autumn the Ministry of Education will decide on generous grants to push the TRISTAN project ahead to completion well before LEP, and give basic research in Japan a big boost. As Professor Ozaki put it "being behind the rest of the world is no way to encourage anybody". □

Synchrotron source

The photon business

THERE is more to KEK than just high-energy physics. Part of the same institute, but virtually autonomous, is the "Photon Factory", one of the world's largest synchrotron radiation light sources.

"Some people have criticized KEK's 'big science' ", said the Photon Factory's director Kazutake Kohra, "but here we do small science, involving very many small groups and quite different from high-energy physics research". With a thousand registered users expected by the end of the year, the "factory" is democratically run; advice comes from users' groups at many universities and in many disciplines.

Full-scale operations at the Photon Factory began in June and are building towards the machine's design characteristics of a stored current of 500 mA at 2.5 GeV — in the same big league as the UK synchrotron radiation light source at Daresbury or the US National Light Source under construction at Brookhaven. The world's first superconducting "vertical wiggler" magnet and an undulator were installed early in the year, and beam lines supplying intense sources of vacuum ultraviolet, soft X rays and X rays are now being opened up. Nine are already in operation, eventually there will be twenty-four. About thirty measuring instruments have been purpose-built, including a range of spectrometers and X-ray scattering and diffraction devices.

The Photon Factory may not, however, give value for money unless new beam lines are opened up more quickly. A slow-down in the flow of funds from the Ministry of Education has now shifted emphasis to persuading users from government institutes and industry to pay for opening of their own lines. Already, Nippon Telegraph and Telephone has constructed one line at its own expense (on the understanding that it will be available to other users for half the time) and the

Electrotechnical Laboratory (supported by MITI) is soon to make a big investment in new facilities.

That industrial users and others from outside the Ministry of Education are involved at all is something of a triumph for the Photon Factory. "Only a few years ago, there was a very strong taboo against letting Ministry of Education facilities be used by industry or government institutes", Kohra said. "Until recently, industry contacts have been at the individual level and there have often been strong feelings against industrial links, particularly at the time of the student struggles."

Even now, industrial use is being limited to ten per cent of beam time, whereas at Brookhaven's 800 MeV synchrotron, industrial users take about 70 per cent of the beam time and have paid for roughly half the facility. Like the Americans, the Japanese industrial users are mainly after an intense source of X rays for testing the lithographic techniques which will make the next generation of very-large-scale integrated circuits.

Although the Photon Factory is in the same class as — or better than — anything the United States or Europe has to offer, Kohra regrets that Japan has not seized the opportunity to leapfrog other nations. Under construction right next door is the 6–8 GeV accumulator ring for the TRISTAN electron-positron collider. To have installed an undulator in that ring would have given Japan far and away the most powerful synchrotron light source in the world, but not enough money could be found. "We'd be given the money if such a ring was already being built in a foreign country, but when there is nothing to catch up with, both the ministry and the scientists hesitate", complained Kohra. "When there are new ideas that haven't been tested, then we should make haste, not hesitate." □

What of the women?

THERE are no women to be found among the academic staffs of either Tokyo or Kyoto University's science faculties. Nor is there a woman among the seventy-two scientists honoured by membership of the Japan Academy.

There are plenty of woman primary school teachers, nurses, medical technicians and pharmacists. But outside a small range of health care and teaching jobs there are few opportunities for women to pursue a career. Science, like law and the civil service, is an area where the barriers to advancement are particularly high.

First of all, only a tiny number of women ever manage to get into a top university. At Kyoto University, for example, women make up a mere seven per cent of admissions; and not because the university has a discriminatory policy but because parents send off only their sons to cramming schools for that essential extra examination tuition.

But even this handful of talented women is unlikely to get far. One exception is Professor Katsuko Saruhashi, until recently director of the Meteorological Research Institute's geochemical laboratory. She points out that only 1 per cent of department heads within government research institutes are women. And taking all university staff — scientific and non-scientific — only around 3 per cent are women.

Professor Saruhashi has been fighting to improve women scientists' prospects for most of her career. In 1958 she helped found the Society of Japanese Woman Scientists. Three years ago she put the money she received on retirement into establishing the Saruhashi Prize, to be given each year to an outstanding woman scientist. The prize is not such a great sum (Y300,000) but it is having the effect of drawing public attention to the work of women scientists and making more women believe they can succeed in science.

Professor Saruhashi believes the lack of child care facilities and poor legislation on women's rights make it very hard for a mother to continue her career. There are many cases, she says, of women who wanted to become scientists and researchers but were forced to give up. For those who do pursue their careers, gaining promotion is an even bigger problem. In almost all jobs, promotion for women ends around the age of twenty-five, the age at which they are expected to marry and become housewives. As a result the average wage for women is only a little over half that for men.

What are the prospects for change? Not so great it seems, for the majority of women profess themselves content with their roles as housewives and mothers. And a recent survey of "intellectuals" revealed that only a little over a third disagree with the statement that "Men should go out to work and women should stay at home". □

Sony's secret

Adapt and survive

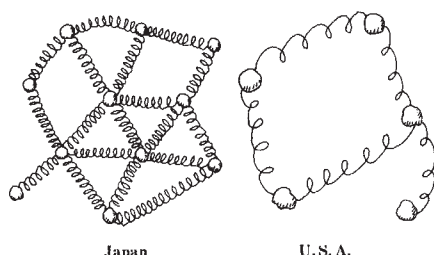
THE legend of the Sony Corporation's foundation is still eagerly repeated. In 1946, the cornerstone of the founder's business was a simple device for cooking rice — a pot marked with the water-level needed for a predetermined quantity of rice and the immersion heater that could be plugged into a lighting socket. At the end of the Pacific war, people snapped them up, and a large slice of the profit was ploughed back into the improvement of the product. Except for the mid-1970s, the company has not since then faltered in its growth.

Makoto Kikuchi, director of Sony's research centre near Tokyo, is thus well placed to explain Japan's rise to the leading position in electronics research. His own research career began in 1948, the year of the transistor, and has taken him through MITI's Electrotechnical Laboratory and the Massachusetts Institute of Technology in the United States to his present position. Kikuchi says that in 1948, his laboratory had only "three or four circuit testers, some pliers and screwdrivers and a couple of ex-army cathode ray oscilloscopes". He is practised at dealing with curiosity about Japan's success. No part of his account attributes magical powers to MITI, or to the spirit of "Japan Inc."

If there has been imitation, it has not been blind. "The sheer technical difficulty of mastering zone melting techniques, for example, prevents learning by imitation. You have to understand the fundamentals before you can even begin."

Kikuchi's formula for success blends sheer hard work and a special brand of "Japanese creativity" distinct from the American equivalent and, most important, particularly suited to electronics research now and in the coming decade.

Kikuchi calls the "sudden inspiration" of an Einstein-like genius "independent creativity", but says the Japanese strength lies in "adaptive creativity" — the ability to make intense concentrated efforts on problems whose general nature has already been defined. These two different styles come from the nature of the two societies. Kikuchi provides the little mechanical model of US and Japanese societies shown below. Japanese society is likened to a large number of small stones bound together by strong springs. American society, by contrast, has heavier stones linked by weaker springs.



Japan

U.S.A.

So "members of Japanese society are in close proximity physically and psychologically and have a powerful influence on one another. A disturbance in one corner of society sets off sympathetic vibrations throughout the entire network of stones and springs. In contrast, in America, greater weight is given to the individual, with correspondingly weaker interactions among its members. When one person moves, it is only rarely that everybody else moves with him."

Kikuchi chuckles over how a rumour some years ago, later found to be totally untrue, that IBM was building a "future system" chip "30 cm in diameter" fired a massive Japanese effort in very large scale integrated circuit research. The false objective was never attained, but the technology of microchip production moved up



Head of Sony's research — Makoto Kikuchi

another notch. Adaptive technology rests in part on the Japanese enthusiasm for the new, what Kikuchi calls the "high mental temperature" of Japan.

But Kikuchi acknowledges that it was relatively easy for Japan to catch up because following behind the United States meant research goals had already been defined and that the mistakes that leaders made could be avoided. One colossal wasted effort by the United States which Kikuchi jokes about was the attempt to develop an electronic refrigerator, intended to use the cooling at semiconductor P-N junctions. A material of exactly the right efficiency could not be found, even though for five years "an enormous number of people made an enormous number of measurements on an enormous number of materials". No electronic refrigerator was ever built.

Catching up was also made easier by the speed with which electronic technology "matured". Kikuchi says that, as more and more became known, new inspired discoveries became less likely, and that even for new developments to succeed, they had

to be judged not only on their own merits but on their match with existing technology.

Kikuchi's message for the United States is that the Japanese way will be even more effective in electronics in the coming decade. The "evolutionary" process at which they excel will keep them at centre stage. Already "we know what needs doing in very-large-scale integrated circuits, optoelectronics and optical fibres, and in these fields Japan will prove unbeatable".

Quite different problems arise in software and the "fifth generation" computer project. Here, Kikuchi says, fundamental new ideas are needed and must be expected to come from the intellectual elites of the United States and Europe, not from Japan where there are "no elites". "In the fifth generation computer project, the true contribution will come from the West, even if the Japanese eventually put it all together."

Can Japan develop strength at "independent" as well as "adaptive" creativity? Kikuchi thinks that difficult, given an educational system that emphasizes "tricks for passing examinations rather than fundamental understanding" as well as enforcing a degree of 'homogeneity' by refusing to stream students according to their ability or to give special attention to the gifted. Kikuchi gives an interesting example: if a Japanese high school student is good at mathematics and poor at music, teachers' efforts will be spent on getting the student up to the average in music, not in cultivating his special talent. "This unwillingness to create an elite is not", Kikuchi stressed, "a matter of policy which can be easily changed, but a reflection on society. The boundary condition is that we all have to survive on top of one another in these small islands."

Kikuchi, then, believes that Japan does have weaknesses which are, in a sense, the result of the same social factors that give Japan its strengths. But what do young people today think?

Like many of his generation Kikuchi's views were shaped by his experience of the terrible conditions at the end of the war, and the early days when Japanese went on humble pilgrimage to learn at American universities. Kikuchi himself feels a profound gratitude for the opportunities he received in America of meeting the world's most brilliant electronics researchers. But the present generation are growing up accustomed to Japan as a world leader and are totally Americanized in their fads and fashions. They feel the difference between Japan and the US is not so great. Kikuchi is not so sure that the individualism he feels as necessary for truly innovative research is any more present in today's youth. He interviews plenty of young people wanting to join Sony — all dressed in identical suits bought from the "interview corner" set up by department stores each year. "Nothing fundamental has changed" he says. □

What Japan (and others) should do

Japan's pre-eminence in so many industries is a just reward for hard work. What are the lessons for others? And what might happen next?

THIS survey of science in Japan is in no sense a formal study. It has been concerned with only some parts of a rich scene and is to a large extent based on what people have said, sometimes in contradiction with each other. Of necessity, an account compiled in such a way must be coloured subjectively, and should be read in that spirit. Similarly, it has openly been influenced by the widespread general interest just now in the peculiarly Japanese success of the past twenty years. Yet even to ask "How has Japan been so successful?" is to presuppose an answer other than "Why not?". (On the other side of the Pacific, the implicit assumption is often that the United States will continue indefinitely to be ideally innovative and ingenious, so that the usual loaded question is "Why has it faltered?"; the same answer, of course, can be used.)

The issue is nevertheless interesting. The subjective explanation already given (see page 355) is that there is nothing magic about Japan's technology, but that Japanese technologists are more willing than others to let markets define their objectives, to regard functional excellence as a necessity in a marketable product — and then to work hard and quickly. Japan should be pleased that this or something like it has become a model for success in the application of technology that others must follow as best they can. But the fact that Japan has not accomplished everything is neither a surprise nor a disgrace, for there are simply not the people to go round. Just as well, it will be said elsewhere.

So why is there so much worry in Japan about the problems that lie ahead? "We must be more creative" is the cry, as if this is something to be accomplished simply by exhortation. No doubt something will result — some managements may be even more ready than now to regard their young recruits as the chief sources of new ideas. But if the cry is serious, there seems no choice for Japan but to strengthen basic science relative to engineering, which means tackling at long last the reform of the universities.

In 1972, *Nature* complained that the government was neglecting basic science in the universities. Materially, things have improved a great deal since then, especially by the creation of institutes such as those at Okazaki (see page 372), yet basic science remains a poor cousin to technology. Students know where the good jobs are, and decide accordingly. Nobody will underestimate the difficulty of correcting

this imbalance. The complaints are several. The pattern of studies at the national universities is too rigidly determined from outside, students are too regimented, the government's influence on the support of research is too detailed, the harnessing of so much research to short-term projects may be good for the economy but is bad for basic science, even sensible major projects such as the building of accelerators, millimetre-wave telescopes or synchrotron sources are so thoroughly chewed over by committees that they rarely break new ground and finally, it is hard to see how, within the system, genuinely new lines of enquiry could flourish.

To remedy all this would require that universities should have more autonomy, more resources for research and more say in how they are used. And Japan's admirable diversity of function within the university system needs to be matched by a loosening of the hierarchy. Tradition and the government's budget officers would be offended, but those would be small prices to pay for an overdue reform.

Other more contentious points arise. The constant rumbling about the terms of trade between Japan and its industrialized partners has been unwisely fanned from the West, but there seems no doubt that there is a core of justifiable complaint. And too many of the defences offered in Japan are based on the assumption that to explain why a problem exists is the same as solving it. The point is important for the technical community as a whole because of the threat to the flow of information. Chauvinism is another worry. The boom in Japan has fostered the use of phrases such as "our technology". By a largely emotional reaction, similar usages are cropping up elsewhere. The tendency is both dangerous and mistaken, implying as it does that technology is an end in itself and not a means to some other. How can it be stopped?

The West has as much to do as Japan itself. Many of the reactions in recent years to the impressive Japanese success have been thoughtless exaggerations. If the reasonable expectation is correct that beneficial technical development will continue to expand, it follows that even the most confident society cannot hope to do everything for itself. Japan will no more be scorned if its ambitions in biotechnology are disappointed than because, now, it is not a major manufacturer of civil aircraft.

But it is a dangerous illusion to suppose

that success means succeeding everywhere. Talk, especially in the United States, about Japan's (or MITI's) search for "world dominance" in computers flatters the fifth-generation computer, which exists only as a loose collection of research programmes, some (or none) of which may be useful. But such talk also begs the question of what this nonexistent machine is meant to accomplish, and of whether those objectives might more easily be met by other means.

In the technological rivalry between Japan and its trading partners, proper if mere competition but pointless if chauvinistic, one serious consideration is too often overlooked — the possible fragility of the Japanese economy. The huge return on exports each year is usually nearly in balance with an equally huge import bill — the cost of paying for raw materials that do not exist within Japan. Since the end of the Pacific War, insecurity engendered by that knowledge has no doubt been a powerful spur to technical innovation. And it must be remembered that Japan's competitors are now themselves getting into better shape. The announcement of the fifth-generation computer project pushed other countries into increasing their own spending on artificial intelligence and expert systems research. Efforts too are being made to learn from Japan's methods for promoting cooperative research among competing companies. Things could go wrong at any time — another increase in the price of oil, the collapse of some major project or company, trouble with a neighbour or a change of government. Such events are not impossible even in prosperous conservative Japan. After all it has happened before. It is only half a century since politicians were in danger of assassination, and only 125 years since Japan was a feudal state. People are cheerful and good-humoured now, but they are not oblivious to the past.

The professional community has a specific part to play. Japan is a long way from most places, so that a sense of isolation is almost unavoidable. Yet, as these pages may have helped to demonstrate, Japanese science is no less a part of the whole than reasonable people would expect. It deserves to be better known, not merely by being published but by being understood in the kind of sympathetic detail common elsewhere. Only professional colleagues can ensure that that happens. □

Thirty years of DNA

After thirty (and a half) years some of the fundamental questions of biology now seem poised for a molecular answer.

"If you are young", said Jim Watson in his opening remarks at the conference organized by *Nature* in Boston, 19–21 September, to recelbrate the thirtieth anniversary of Watson and Crick's paper on *A Structure for Deoxyribose Nucleic Acid*, "there is really no option but to be a molecular biologist". And so it will be for at least the next twenty years, predicted Watson, by which time, the molecular approach should have solved the fundamental questions of embryology and differentiation. In Watson's view the answers will come from studies of cancer, a view that seemed easier to sustain at the start of the meeting than by the time protagonists for lymphocytes, erythroid cells, mice, fruit flies, slime moulds, nematodes and plants had had their say.

One question that looks close to being answered in *Drosophila* is that of the character of the switches that turn individual genes on and off at appropriate times in development. The presumption is that the switches are encoded in the DNA that flanks the coding region of genes, and Gerry Rubin (University of California, Berkeley) described an experimental approach that is close to identifying them. It involves microinjecting *Drosophila* embryos that have a genetic deficiency of an enzyme with the gene for the enzyme, carried in a "P-element" vector. In Rubin's experiments, the enzyme was xanthine dehydrogenase, encoded in the *rosy* locus of *Drosophila*. Mutations in the *rosy* locus lead to loss of red pigmentation in the eye.

Rubin has developed many lines of red-eyed *Drosophila* from *rosy* mutants by integration of the *rosy* gene into embryos, thereby proving that the correct time and place of xanthine dehydrogenase production can be restored. Moreover, this opens the way to experimental identification, by their removal, of those parts of the gene, or more likely its flanking regions, that are responsible for its correct expression.

Surprisingly, in no two lines had the xanthine dehydrogenase gene become inserted into the same chromosomal position and yet in all but two cases normal eye pigmentation was restored. However, in experiments involving the effects of the interactions between the *white* locus and *zeste* on eye pigmentation, Rubin has found a much greater sensitivity to the site of integration. Why some genes are much more sensitive than others to their position

on chromosome, is now amenable to a molecular answer.

So, too, may molecular answers be forthcoming to the question of how spatial and positional information are delivered and sensed in *Drosophila*. Gary Struhl (Harvard), set the scene by describing the four classes of genes that successively control the proper development of the segments of a *Drosophila* larva. Initial clues come from 15 genes that control overall body polarity. Thereafter two separate classes control segmentation of the body. Finally genes of the *bithorax* complex take over with, it is proposed, more *bithorax* genes being switched on,



Francis Crick (left) with John Maddox, *Nature* Editor.

the more posterior the segment. The suggestion that the *ESC* gene then regulates the expression of *bithorax* genes in each segment is supported by Struhl's data and like genes of the *bithorax* complex *ESC* is a ready target for cloning.

Another organism favoured by developmental biologists is the slime mould, *Dictyostelium discoideum*. The initial stimulus to its developmental cycle — a high density of the amoeboid cells — induces the expression of a set of genes. Harvey Lodish (MIT) described the identification of one of the gene set as that for actin. Another, which he said belonged to a family of about 250 closely related genes, was interesting for the fact that it was preceded by a short sequence of DNA that differed in only one of its 15 bases from the sequence that precedes a family of *Drosophila* genes that are induced by heat treatment of the flies. Indeed, Lodish said, heat was also able to induce transcription of both the slime mould genes. Further investigation of several members of the 250-gene family showed them to have the characteristics of transposable genetic elements, raising the possibility that transpositions underlie the developmental programme of the slime mould.

The nematode, *Caenorhabditis elegans*,

is another simple organism studied by developmental biologists under the influence of Sydney Brenner (MRC Laboratory of Molecular Biology, Cambridge) who described its neuronal cell lineage. Armed with that blueprint, and with mutants and molecular genetics, man, the genetic mechanic, may yet become man, the genetic engineer, he implied. Mechanic or engineer, Howard Goodman (Massachusetts General Hospital) is intent on improving, in the anthropomorphic sense, the genetic character of plants. One of his interests is to transfer genes for herbicide resistance into alfalfa, reducing its susceptibility to herbicidal spraying. Goodman described an experimental investigation of a variant alfalfa cell line that displays resistance to the herbicide PPT, an inhibitor of glutamine synthetase. Its resistance appears to be the result of an overproduction of glutamine synthetase due to amplification of the gene for the enzyme. Whereas that may constitute the first example of gene amplification in plants, it is disappointing in an applied sense for which the requirement (for gene transfer) is a gene encoding a variant enzyme whose PPT-resistance is the result of structural alterations.

The study of the genetic rearrangements that underlie the development of the B cell, with its well defined pathway of development culminating in a cell almost wholly devoted to antibody production, has been enormously productive of both fundamental facts and rococo detail. In the rearrangement of the immunoglobulin light chain gene segments that brings together one of the 50 or so variable regions with one of the five junction regions, there is suggestive evidence that the DNA between the joined segments is not deleted, but inverted. David Baltimore (Whitehead Institute), described experiments involving transfection of PD cells with a retroviral vector carrying the joining segments and a variable segment of a kappa light chain gene in such a way that reorientation of the variable segment during rearrangement turned on a selectable marker gene. That the system works reveals that the relative position of the segments on the chromosome cannot be important to the joining machinery and may make it possible to develop systems for bringing together designated variable and joining segments to order.

The type of retroviral vector used in these experiments has been developed both

in the laboratory of Howard Temin (University of Wisconsin) and by Richard Mulligan (MIT). Mulligan outlined the advantages of retroviral vectors over others particularly when the aim is to obtain stable integration of a gene in a high proportion of a population of cells. Moreover, he held out the prospect of being able soon to extend the host range of retroviral vectors from their species of origin (generally the a mouse) to other species; by such means it may become possible to correct genetic defects of human haematopoietic stem cells. So, too, does Rudy Jaenisch (Hamburg) plan to use a retroviral vector to correct a genetic defect — in his case, a mouse strain that he has experimentally produced by the introduction of a retrovirus into mouse embryos. In this particular strain, the retrovirus has become inserted into the $\alpha 1(I)$ collagen gene, inactivating it and causing a lethal homozygous mutation.

One of the features of retroviruses that makes them into good vectors is that they have a "long terminal repeat" sequence at both ends that is capable of enhancing the activity of nearby genes. The discovery that a root cause of bursal lymphomas of chickens is the insertion of a viral long terminal repeat next to the cellular *myc* proto-oncogene in such a way as to enhance its activity, was a key event in focusing so much of cancer biology onto oncogenes in recent years, another being the identification of transforming *ras* genes in about 20 per cent of human tumours. Robert Weinberg (MIT) described the meeting of these two ways in the ability of *myc* and *ras* genes, acting in concert, to transform primary cells to an extent that neither alone could do.

Parts of the gene enhancing region of a DNA tumour virus, SV40, occupy the attention of Alex Rich (MIT) for a different reason — namely their propensity to form Z-DNA. This is the left-handed form adopted by stretches of DNA in which purines alternate with pyrimidines, particularly under the influence of a high degree of negative supercoiling — as elegantly shown in experiments described by J. Wang (Harvard). Rich outlined experiments by which it had been demonstrated that the gene regulatory region of SV40, when negatively coiled, contained three 8-base pair stretches of Z-DNA, one in each of the 72-base pair tandemly repeated sequences and one close by. Experimental removal of one of the 72-base pair repeats leaves a fully active virus. If, however, that virus is subjected to site-directed mutagenesis in such a way as to disrupt the pattern of alternating purine/pyrimidine bases in both remaining 8-base pair stretches, the resultant virus can no longer either replicate or produce T-antigen, adding circumstantial evidence to support Rich's proposal that stretches of Z-DNA, held in that conformation by specific binding proteins, act as physiological recognition sites for RNA

polymerase. It will, however, be a very long time before as much is known about the interaction of Z-DNA with its binding proteins as Mark Ptashne (Harvard) already knows about the binding of prokaryotic repressor proteins to their operators. From the crystal structures of repressors it has been possible to model accurately their interactions with DNA and to confirm the models with mutant and hybrid repressors. As diffraction data emerge from a co-crystal of the repressor of bacteriophage 434 with a 14-base pair operator-like polynucleotide, a direct test of the models becomes increasingly possible.

As it happens, it is also a bacteriophage regulatory site that has been of recent interest to Tom Maniatis (Harvard) but for a very different reason. Because of the detailed analysis of the molecular basis of individual cases of β -thalassaemia in his and other laboratories, it has become clear that one cause is the occurrence of



Wally Gilbert (left) with Sydney Brenner.

mutations that either directly or indirectly affect the normal splicing out of the two introns of the β -globin gene. Most often, a mutation that conceals one of the genuine splicing recognition sites allows other, "cryptic" recognition sites to be revealed. Maniatis described the beginnings of an *in vitro* system that should help the mechanism of splicing to be understood. Crucial to the system is the availability of large amounts of pre-messenger RNA for β -globin as a substrate. Maniatis explained how to produce that by means of attaching a *Salmonella* bacteriophage gene promoter to a β -globin gene in a plasmid and turning on expression with a bacteriophage-specific RNA polymerase. The splicing substrate produced in that way has now been used to develop a system of *in vitro* splicing, stimulated by a HeLa cell nuclear extract, that is reasonably efficient and, in part, accurate.

The β -globin gene transcripts, with their two introns, present a simple problem for the splicing machinery, whatever it is, compared with the transcripts of the vertebrate $\alpha 1$ - and $\alpha 2$ -collagen genes, each of which contains around 50 introns (Helga Boedtker, Harvard). A comparison of collagen genes across vertebrate species and between vertebrates and invertebrates drew Boedtker to the view that introns are

relatively free to vary in size but not in position; the paucity of introns in invertebrate genes probably reflects the evolutionary loss of introns from an ancestral gene — a view supported, in general, by Wally Gilbert (Biogen).

Although almost all vertebrate genes have introns, the α -interferons are an exception, that being one of the reasons why Charles Weissmann (Zurich) was first able to produce an α -interferon in *Escherichia coli*. He described more recent systems for the secretion of α -interferon from *Bacillus subtilis* cells and its production in hamster cells, as well as the production of various hybrid and mutant α -interferons whose relative biological potency is to be explored.

David Goeddel (Genentech) described the production of γ -interferon genes inserted into "cos" and yeast cells and preliminary evidence of the efficiency of mouse γ -interferon from "cos" cells as an antitumour agent.

While recombinant DNA techniques are producing new therapeutic agents they are also yielding new or improved prenatal diagnostic tests. In particular Y.W. Kan (University of California San Francisco) described the use of a synthetic oligonucleotide probe for the mutation that converts into a stop codon, the codon for glutamine-39 of β -globin in many cases of β -thalassaemia. He also described a method of prenatal sex determination that is faster than karyotyping and makes use of a Y chromosome specific DNA probe.

Recombinant DNA techniques are also beginning to pay dividends in the neurosciences, both in such relatively well-defined circumstances as the exploration of genes for the peptides that control the egg laying behaviour of *Aplysia* (Jim Jackson, Columbia University) and in such relatively ill-defined territory as the exploration of what is encoded by brain specific messenger RNAs of the vertebrate (Floyd Bloom, Salk Institute). Among the messenger RNAs examined by Bloom and his colleagues are several that share an identical 62-base pair "ID" sequence within an intron and one whose sequence has revealed potential new peptides, synthetic forms of which — and antibodies thereto — have been used in electrophysiological and immunohistochemical experiments to explore the role of the peptides in the brain.

For Bloom, a classical-turned-molecular neurobiologist, as for Watson, that is the approach to take. For Francis Crick (Salk Institute), whose thoughts have turned from molecular biology to classical neurobiology, the way ahead is less certain but must include the ability to make lesions that disrupt very specific brain functions. Nevertheless, Crick admitted that, contrary to his expectations, he found himself sufficiently interested in the intensely molecular biology of the meeting's talks to sit through them all.

Peter Newmark

Oceanography

A clever answer to a simple question

from Arnold L. Gordon

AMID the complexities of modern science there are still some simple but important questions. One such question, 'Why is no deep water formed in the North Pacific?', is posed and answered in a highly readable article by Bruce Warren which recently appeared in the *Journal of Marine Research* (43, 327; 1983).

In the North Atlantic, surface water cools in winter and, as its density increases, sinks to the ocean bottom. There it spreads and flows southwards until, around the latitude of Bermuda, it meets and rises over an even colder and denser bottom-water mass generated in the Antarctic. In the high northern latitudes of the Pacific, however, a similar process is not seen; surface water does not sink to great depths.

The immediate explanation for the difference is that Pacific surface seawater is much fresher than that of the Atlantic (32.7‰ salinity for the North Pacific against 34.9‰ for the northern North Atlantic; see the figure); its density is thus so much less than the water it overlies that it cannot sink to the ocean bottom, even when cooled to freezing point. The real question, then, is why the contrast between surface- and deep-water salinities is so much greater in the North Pacific than in the North Atlantic.

North Atlantic surface water is not only more saline than North Pacific water, it is also far warmer. As Warren demonstrates, these two factors are not unrelated. He carefully shows that a 'logical' common

first guess in attempting to explain the salinity difference is wrong; that is, the Pacific is fresher because it receives more freshwater by precipitation and continental run-off. In fact the North Atlantic receives more freshwater than the North Pacific. The answer lies instead in the difference in evaporation rates. The evaporation rate from the North Atlantic surface is more than twice that from the North Pacific surface. In the Pacific freshwater input accumulates, tending to depress surface salinity and in turn inhibit convection.

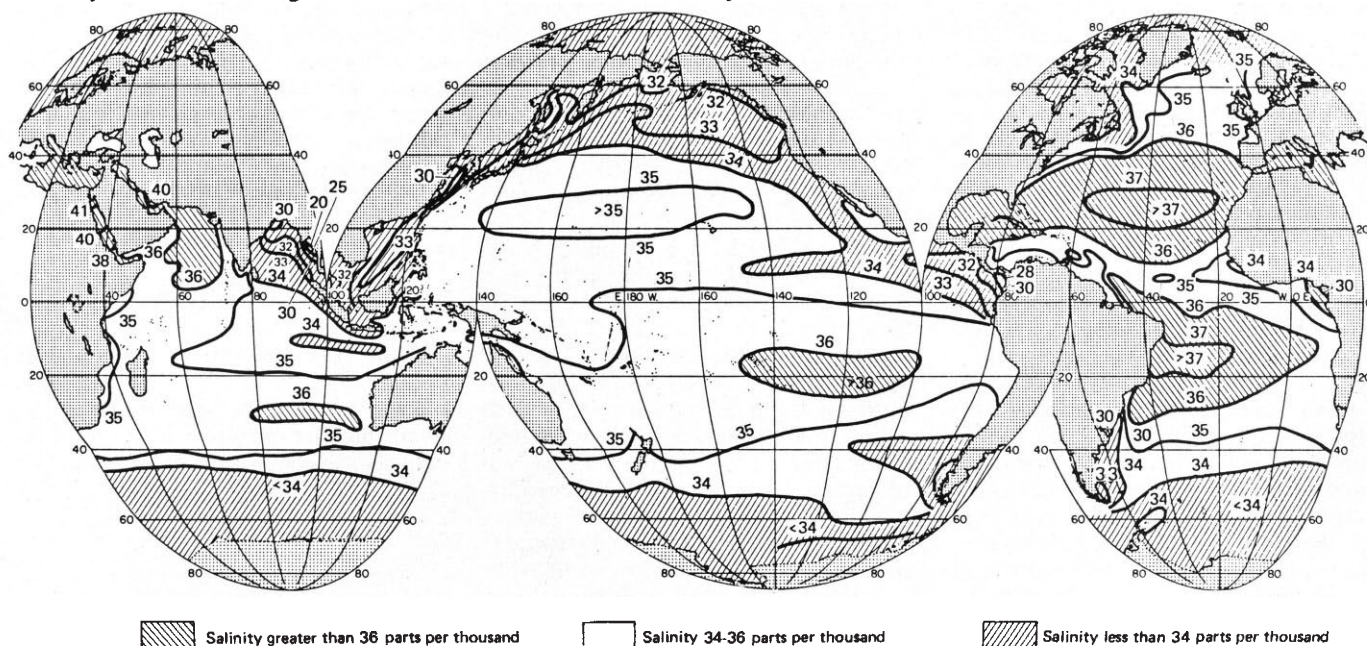
Warren shows that the low evaporation values can account for the very stable surface water 'cap' of the northern North Pacific with the aid of a non-diffusive steady-state salinity budget model. Indeed, the very lack of deep convection increases the residence time of surface water in the North Pacific, reducing the possibility of inflow of saltier subtropical surface water from the south. Conversely, in the North Atlantic the loss of surface water into the abyssal ocean brings about a net northward drift of salty subtropical surface water.

That brings us to the next and more fundamental question: why is evaporation so low in the North Pacific? Warren very carefully shows that the reduced evaporation is due to the low surface-water temperatures of the northern North Pacific. Cold water, by decreasing the specific humidity of the air in contact with the ocean, reduces evaporation. Warren

notes that this difference is not due to decreased winds over the North Pacific, it is definitely a thermal effect. The coolness of the northern North Pacific surface water is a consequence of size and isolation of the subpolar gyre. The maximum westerlies of the wind field are further south in the North Pacific than in the North Atlantic, limiting the effectiveness of the wind to transfer warm subtropical water northwards. The more southerly position of the North Pacific maximum westerlies may be a response to larger-scale climatic factors, "... perhaps even the planetary orography as well".

"It seems bizarre at first thought that one can reduce the density of the water in a region by decreasing its temperature", Warren says, but he provides a simple model to aid in understanding this situation. It is here that Warren presents a very nice linkage between latent and sensible heat exchange.

Using a linear relation for the specific humidity of saturated air as a function of temperature, a simple equation for temperature and for salinity is derived as a function of volume flux of water through the system, initial temperature and salinity and atmospheric heat and freshwater forcing. A consequence of this treatment is a determination that the inflow flux into the northern North Pacific surface-water reservoir is $4 \times 10^6 \text{ m}^3 \text{ s}^{-1}$ with an initial temperature of 5°C , which matches that of the Ekman-induced upwelling water in the subpolar gyre. While subsequent warming of the upwelled water contributes to low surface-water salinity, most of the surface-water density is attributed to the reduced salinity by accumulation of precipitation and run-off. Warren notes that in the North Atlantic a similar approach would show that the surface-water density increase due to temperature decrease 'wins',



Surface salinity of the world ocean (values in parts per thousand). Taken from *Oceanography a View of the Earth*, 3rd edn, by M. Grant Gross and published by Prentice Hall, New Jersey.

allowing deep-reach convection.

Warren's article is important in that it provides in clear terms a satisfactory answer to the question of why no deep water forms in the North Pacific. In addition, it provides a rich source of literature in atmospheric freshwater balance and on large-scale North Atlantic and North Pacific oceanography. It also clearly

demonstrates that the climatic impact of the ocean cannot be fully realized within the thermal field. Salinity and freshwater forcing must be considered in understanding the three-dimensional circulation pattern.

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Deep-sea drilling project Leg 93

The continental rise off North America

from the Leg 93 staff

DEEP-Sea Drilling Project Leg 93 (May–June 1983) achieved, for the first time, deep penetration of strata beneath the continental rise off the east coast of the United States. This exploratory drilling along the eastern passive margin at the interface between continental and deep oceanic environments revealed an extensive Lower Cretaceous sandy deep-sea fan complex that proved to be a proto-oil reservoir and shows evidence of an asteroidal impact.

The drilling was carried out in two areas: on the lower continental rise some 270 nautical miles (n.mi.) east of Cape Hatteras, North Carolina (DSDP Site 603); and on the upper continental rise about 100 n.mi. south-east of Atlantic City, New Jersey (DSDP Sites 604, 605).

Three holes at Site 603 penetrated to a total depth of 1,585.2 m and provided nearly continuous core coverage throughout the entire Valanginian to Lower Pleistocene section. The 1,025.26 m of total sediment recovered is a DSDP record for any one site. The major discovery here was the intersection of an Upper Hauterivian–Barremian passive margin deep-sea fan complex. At a sub-bottom depth of 1,119 m, sand and sandstone turbidites of the fan complex comprised about 47 per cent of the section over the next 218 m of section. Largely unconsolidated terrigenous sands (disturbed by drilling) constituted nearly all of the recovered sediment over a 39-m interval at the top of the sequence. Below that, individual turbidites ranged up to 2 m in thickness and were dominated by sub-angular quartz with abundant feldspar, mica, heavy minerals, opaques, wood fragments (locally up to 20 per cent), glauconite and shallow-water bioclastics. They exhibit the entire range of Bouma textures with the basal sequences dominant (Ta to Tc), including intraclast-rich debris flows that contain plastically deformed blocks of pelagic sediments up to 25 cm across.

Interbedded with the sand turbidites are black claystone turbidites and intervals of pelagic chalks or limestones. 'Black shale' turbidites are common and become the dominant lithology in the overlying 106-m-thick Aptian–Albian beds where their organic contents range from 4.1 to

13.6 per cent of the rock. These and intermittent sand turbidites persist well into the Upper Cretaceous where claystones are the dominant lithology both there and in the overlying more than 1,000-m-thick Cenozoic section.

Source rock, reservoir rock and cap rock, usually considered diagnostic of petroleum accumulation, are present at Site 603. The Site itself lies at an oceanic depth beyond present technical capabilities for petroleum exploitation and no mature hydrocarbons were encountered there because of the shallow burial depth. A reassessment of drilling results from DSDP Site 534 to the south (off the Bahama Banks) and the recently published United States Geological Survey (USGS) seismic study off the Georges Bank to the north, however, indicate that the Lower Cretaceous fan complex could be extensive, perhaps part of an apron of clastic-rich sediments of this age along the lower continental margin of eastern North America. Where other geological conditions are more favourable, this clastic sequence might have petroleum potential.

The Cape Hatteras deep-sea fan complex was emplaced 115–130 Myr ago, at a time when large river deltas prograded across the continental shelf of eastern North America. A coeval outbuilding of terrestrial deltas occurred in the southern Wessex Basin of England (Wealden Beds). Leg 93 drilling showed that the deltas along the eastern seaboard of the United States reached the shelf edge and spilled their loads into the deep-sea environment to build a fan complex extending well beyond the foot of the continental slope. This calls into question two widely held notions: that sea levels at this time were rising; and that the outer continental shelf along the mid-Atlantic states during that period was fringed by barrier reefs which had been present since Jurassic times. The reefs and rising eustatic sea levels would have tended to trap land-derived sand in basins along the continental shelf. The Leg 93 results prove otherwise and support a suggestion contained in a USGS seismic study of the New England area that the outpouring of terrestrial clastics must have overwhelmed the

outer-shelf carbonate environment. Reef development would have been impossible because of the turbid water environment. A relatively continuous 619-m Middle Maestrichtian – Middle Eocene section at Site 605 yielded an expanded Cretaceous–Tertiary boundary sequence. There is no obvious lithological expression of the boundary in the olive gray marly limestone; but the lowermost Danian sediments (planktonic foraminiferal and calcareous nannofossil Zones P1A and CP1A respectively) show a marked rise in the percentage of terrigenous silt and glauconite. Assuming that the silt and glauconite are shelf-derived, this may indicate a drop in sea level at the time of the Cretaceous–Tertiary boundary event. Although of short duration, this basal Tertiary sea-level drop is apparently more pronounced than that noted on the Vail sea-level curve, and could be taken as evidence that an asteroid-sized body did hit the ocean, clouding Earth and causing the massive extinctions associated with the terminal Cretaceous event.

A sharp increase in biosiliceous material associated with a 152-m-thick Lower–Middle Eocene chalk at Site 605 is attributed to upwelling caused by the incursion of the Labrador Current into this area. The shipboard scientists estimate that this current was initiated at around 49–50 Myr.

Attempts to document the Oligocene erosional event associated with Reflector A^u at Site 604 off New Jersey were frustrated when massive uppermost Miocene debris flows and turbiditic sands were encountered which could not be penetrated by *Challenger's* drill. Interestingly, however, the Miocene debris flows contained biosiliceous silty claystones, silts, and sands and conglomerates with quartz pebbles and displaced blocks of Eocene chalk up to 50 cm across. The debris flows and their associated turbidites were emplaced as the result of a major drop in sea level which occurred during the Messinian, a time of severe glaciation on the Antarctic continent. At that time rivers again advanced to the shelf edge to spill their loads while subaerial and current erosion largely denuded the shelf and upper slope of the eastern seaboard of upper Miocene sediments.

Lateral and vertical changes in the types of sediment found in the eastern North Atlantic are far more rapid and complex than in the more seaward environments traditionally explored by deep-sea drilling. Deeper holes will be necessary to understand fully the important province where deep-sea and continental realms meet. □

The Leg 93 staff included J. van Hinte, Co-Chief Scientist (Vrije Universiteit, Amsterdam); S. Wise Jr, Co-Chief Scientist (Florida State University); B. Birt (Open University, UK); J. Covington (Florida State University); D. Dunn (DSDP, Scripps Institution of Oceanography); J. Haggerty (University of Tulsa); M. Johns (Texas A&M University); P. Meyers (University of Michigan); M. Moullade (Université de Nice); J. Muza (Florida State University); J. Ogg (University of Wyoming); M. Okamura (Kochi University); M. Sarti (Università di Ferrara); U. von Rad (Bundesanstalt für Geowissenschaften und Rohstoffe, Hannover).

Continental evolution

The European Geotraverse

from Derek J. Blundell

THIS month sees the start of the European Geotraverse's first major experiment — a deep seismic survey of the lithosphere from northern Italy to Tunisia. Over the next seven years the European Geotraverse Project will study a swathe of ground some 100 km wide and 4,000 km long from the North Cape of Norway to Tunisia (see the map) and will provide a great increase in our knowledge of the tectonic evolution of continental regions of the Earth.

The European Geotraverse Project was initiated two years ago by Stephan Mueller, Professor of Geophysics at ETH, Zurich. Geologists and geophysicists from most of the European countries have been brought together to help formulate the project and support has been received from the European Science Foundation.

Continental regions are much less well understood than the relatively young lithosphere underlying the present oceans of the Earth. They contain what remains of the main time span of the Earth's history, and are composed of a mosaic of structurally characteristic tectonic provinces formed at various times through their history, the oldest Precambrian provinces more than 3,000 Myr old acting as nuclei around which successively younger ones have formed. The evolution of the continental lithosphere is complex, with successive thermal and deformational episodes superimposed, and the nature of the processes involved remains obscure.

Europe is particularly suited to a major project for it is made up of a number of tectonic provinces ranging in succession from the oldest Precambrian areas of Scandinavia to the currently active area of the Mediterranean. The Geotraverse Project will provide a continuous integrated study of sufficient scale to give new information about both vertical and lateral variations of the whole lithosphere, both within and between adjacent provinces. Equally important, because the provinces occur in succession the project will provide an opportunity to follow the progression of tectonic activity over time and thus learn about the dynamics of the lithosphere.

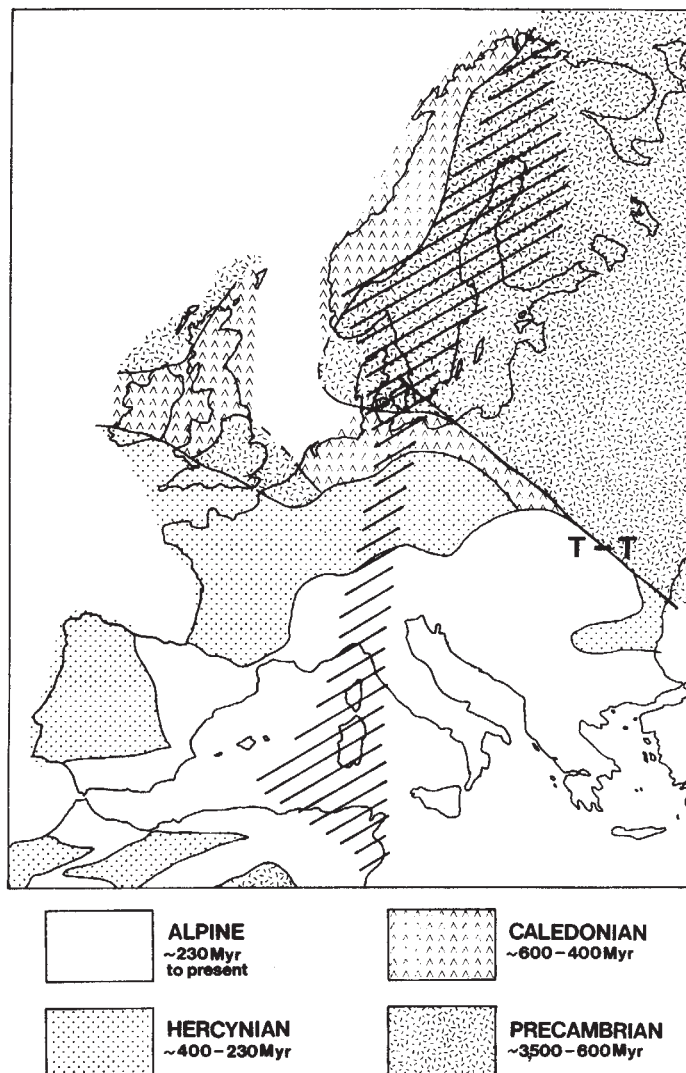
For practical purposes the Geotraverse is divided into three segments (see the figure): the northern one covering the Precambrian provinces of Fennoscandia; the central one crossing the Hercynian realm of Central and Western Europe; and the southern one traversing the Alpine-Mediterranean region. It is located to take advantage of existing information, especially from the larger-scale geophysical measurements

such as the Fennolara seismic experiment which has provided a regional pattern of the crust and upper mantle structure underlying Scandinavia.

Detailed studies will be made to attack key problems and broader-scale measurements will seek out regional variations. Geophysics features strongly on the experimental side to provide information on deep structures and on dynamic problems but geological and geochemical expertise will also be vital, especially in gaining an overall synthesis. The results will be integrated into a north-south section through the crust and upper mantle of Europe which will provide the basis for a reconstruction of the evolutionary development of the various tectonic provinces and their mutual interaction.

Projects include a multidisciplinary

study of the contact zone (the Tornquist-Teissyre Line) between Precambrian and Hercynian Europe, covering southern Norway and Sweden, Denmark and northern Germany in 1984; mapping the full length of the Geotraverse with gravity, magnetic and geoelectrical methods; seismological observations and multidisciplinary studies of Corsica, Sardinia, the Southern Alps, the central European Hercynian province and the border regions between different tectonic units within it, the Sveconorwegian Caledonides and the Baltic Shield. Also within the programme are two experiments off the line of the Geotraverse but vital to it: a study of the anisotropy of seismic-wave propagation in the upper mantle, known to be present along the Geotraverse beneath southern Germany, but which can be studied better across the Iberian Peninsula; and a wide-aperture seismological array (NARS) which is being set up along a great circular route between Goteborg and Malaga to provide seismic surface-wave information with which to model lithospheric structure. □



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The European Geotraverse (hatched area) crosses the major tectonic provinces of Europe. T-T is the Tornquist-Teissyre Line.

Astronomy

The black cloud

from Joseph Silk

THE Universe is mostly made of dark practically invisible matter which astronomers have not seen directly but whose existence can be inferred from its gravitational effects. There are well documented cases of galaxy clusters where 90 per cent of the inferred mass is unseen. In individual galaxies, including our own Milky Way Galaxy, substantial amounts of dark matter are present. In this week's issue of *Astrophysical Journal Letters* (273, L1), Cornell University astronomer S. E. Schneider and colleagues report the discovery of a most extraordinary cloud of dark matter — in which there are no detectable stars at all — in the constellation of Leo. This object offers a unique opportunity to study an isolated sample of almost exclusively dark matter.

Ordinarily, in a spiral galaxy such as our own System, stars dominate the inner region. There is also some gas, detected as neutral hydrogen via the 21-cm radio emission line and also via rotational transitions of various molecular species. The gas, like the nearby stars, rotates with respect to the centre of our Galaxy. Obscuration by particles of interstellar dust prevent our seeing the more distant stars, but the gas can be studied at 21-cm wavelength throughout our entire Galaxy. The rotation velocity of the gas is found to be relatively constant with increasing distance from the galactic centre. In our own Galaxy, the velocity is about 250 km s^{-1} : the Sun takes some 200 Myr to complete a single orbit. Now, in the Solar System, the rotation speed of more distant planets decreases. We interpret this as simply due to the reduced gravitational pull from the more remote Sun. A space probe sent away from the Sun will decelerate; in contrast, a similar probe moving towards the outer galaxy would not slow down.

The lack of decrease of rotational speed with distance from the galactic centre means that the gravitational potential energy remains approximately constant. Most of the mass of our Galaxy must be distributed in such a way that the mass M enclosed within a sphere must increase proportionately with the radius R of the sphere. The rotation speed V will then stay roughly constant, since $V^2 = GM/R$, where G is Newton's constant of gravitation.

Within the circle defined by the Sun's orbit around the Galaxy, the total mass enclosed can be readily made up from ordinary stars. This can be seen directly in the ratio of mass to luminosity in the blue spectral region, which takes a value of about 3 solar masses per solar luminosity for the inner sector of our Galaxy. Such a ratio is found for a distribution of stars whose mean mass is about half that of the Sun, as is indeed the case for our Galaxy. A similar

situation holds for the visible regions of other spiral galaxies. Only in the outermost regions of a spiral galaxy, where the surface brightness is decreasing rapidly, yet the cumulative mass is inferred from the rotation measurements still to be growing, does one find a ratio of mass to luminosity that is larger than can be interpreted as due to ordinary stars. For the galaxy as a whole, the ratio may be as great as 10 or 20 solar masses per solar luminosity, but of course locally where there is little light and much mass, it attains even larger values of 100 or more. It is such large values of mass to luminosity that have led astronomers to postulate the existence of exotic objects, such as black holes or very low-mass planet-like stars, in large numbers.

The discovery of the black cloud in Leo is of considerable importance, for it signifies the first detection of a system containing dark matter and little else. A large isolated cloud of neutral hydrogen was discovered serendipitously during a 21-cm survey of galaxy groups conducted at the Arecibo, Puerto Rico, radio telescope — the largest telescope in the world. There are several groups of galaxies in Leo, but the cloud is not associated with any visible galaxy.

The detection of line emission at the wavelength of 21 cm that characterizes the spin-flip electronic transition in atomic hydrogen allows the velocity of the cloud to be deduced from the wavelength shift relative to the wavelength for emission from atoms at rest. The whole cloud has a systematic velocity away from us of $\sim 960 \text{ km s}^{-1}$. The Hubble law relates galaxy distance to velocity, due to the expansion of the Universe, and from this we can deduce that the cloud is at a distance of 10–20 Mpc.

The total mass of hydrogen gas can now be inferred from the intensity of the 21-cm emission: it is found to be between 8×10^8 and 3×10^9 solar masses, the former value applying if the cloud is at a distance of 10 Mpc. Unfortunately, there is great uncertainty in the estimation of the gas mass. At the low density inferred for the intergalactic cloud, collisional excitations may not fully populate the upper level of the hydrogen atom from which the emission arises. This means that, because of inefficient excitation, as much as an order of magnitude more hydrogen may actually be present than is indicated by the emission line.

The width of the 21-cm emission feature provides the most noteworthy information about the gas cloud, however. It is a measure of the internal velocity field, presumably predominantly due to rotation, of the gas, and from it, together with the measured diameter of about 100 kpc, we may infer the total mass. This mass must be present in order for the cloud to be held

together by gravity. The inferred gravitational mass is at least 2×10^{10} solar masses, and more likely to be about 10^{11} solar masses. This mass may be as much as 10 times larger than the mass of hydrogen. There appears to be no underlying galaxy, at least with a luminosity of more than about 1 per cent of that of our own Galaxy.

A search on a deep photographic plate of this region by E. Kibblewhite (University of Cambridge) has failed to reveal any galaxy with a brightness in excess of about 27th magnitude (in the red) per square arc second: this sets a lower limit of at least 30 on the ratio of total mass to blue luminosity for the black cloud. This is an intriguing result, for it seems to show that dark matter may accumulate not just in the relatively rare rich clusters, where mass-to-light ratios of 200 or so are often inferred, but also in sparsely populated regions of space. Indeed, the mass-to-light ratio of the black cloud could be infinite, since no underlying galaxy has been detected. A more relevant ratio may be that of dark matter to ordinary matter, in this case hydrogen gas, which is likely to be large: the most probable value is about 5, but a value as large as 100 cannot be ruled out.

In other systems where we have estimates of total mass, ranging from spiral galaxies to galaxy groups and clusters, the ratio of dark to ordinary matter in stars and gas is usually between 3 and 10. The intergalactic cloud in Leo is in accord with this, suggesting that it is likely to be an isolated self-contained sample of the cosmos, with a universal ratio of dark to ordinary matter prevailing over scales from tens to thousands of kiloparsecs. The intergalactic cloud in Leo may provide us with the first isolated sample on galactic dimensions of dark matter without the contamination of stars. The lack of luminous stars is a surprise; a typical spiral galaxy contains a factor ten more mass in stars than in gas. Somehow, nature seems to have inhibited star formation in the Leo intergalactic cloud.

The potential cosmological significance of this observation is considerable, especially if a large ratio of dark matter to hydrogen is confirmed. For we know that matter in known forms, including stars and gas, falls short of the critical closure density, above which the Universe would be destined to be finite and eventually recollapse, and below which it must be infinite and expand forever, by a factor of, indeed, about 100. If representative of the mean density distribution, the dark matter in the Leo intergalactic cloud provides enough mass to account for about 10 per cent of the closure density.

These are sufficiently dramatic implications that one is well advised to look for any loopholes. The main danger is that the cloud in Leo may not be an isolated gravitationally bound structure. Perhaps the observed gas is held together not by internal gravity but by the pressure of a hotter more diffuse intergalactic medium. Such intergalactic clouds have been predicted by

theories of galaxy formation: hitherto, none as massive as the Leo cloud has been found. The arguments marshalled against this possibility are that, first, no extended X-ray emission characteristic of such a hot medium has been found near the cloud; and second, the velocity structure of the cloud is reasonably regular and the cloud possesses a fairly well defined outer boundary. Such a sharp edge is not expected if

the cloud is a transient object that is being dispersed by its own internal motions. The evidence may not yet be overwhelming, but the black cloud in Leo could turn out to provide information of immense significance for cosmology, as well as for theories of galaxy formation. □

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Cell biology

Dynamics of mitosis

from N. Paweletz

SCIENTISTS researching into the process of cell division recently had the opportunity to exchange novel partly unpublished results and other information at a workshop* held at University College London. The main conclusion to emerge from the meeting was that the idea of a single mechanism for chromosome transport during mitosis would have to be abandoned in the face of evidence for a variety of different mechanisms.

Progress in methods and procedures developed and elaborated since the last workshop on mitosis in 1977 in Heidelberg is striking. The latest results have been obtained by means of microinjection, fluorescence techniques, isolation and characterization procedures, autoimmune antibodies, genetic manipulation, and light and electron microscopy.

A long-standing issue of central importance is the mechanism of chromosome movement. Are filamentous structures involved? Previously, since these structures had escaped detection, they were not taken into consideration. Now K. McDonald (University of California, Berkeley) has clearly demonstrated the presence of microfilaments and 10-nm filaments in the spindle mostly in close association with microtubules (MTs), by a clever combination of fixation and stabilization methods. Future thinking on the mechanisms of chromosome movement will have to take this finding into consideration.

J. Tucker's (University of St Andrews) careful ultrastructural analysis provided a possible explanation for the exaggerated elongation of the dividing micronucleus in *Paramecium*. MTs initially lying beneath the nuclear envelope become incorporated into the spindle as anaphase proceeds. At the same time, MTs increase in diameter from 24 to 30 nm. U. Eichenlaub-Ritter (Ruhr-University, Bochum) confirmed Tucker's results in a different ciliate, also assuming a sliding mechanism for anaphase B (spindle elongation). She showed that at metaphase MTs are composed exclusively of 13 protofilaments, whereas during the final stages of spindle elongation MTs with 13, 14 and 15 pro-

tofilaments coexist with the majority having 14. These important findings indicate a spatio-temporal correlation between function and number of protofilaments and suggest that changes in the population of MTs occur during mitosis. This assumption was supported by A. Lambert's (University of Strasbourg) demonstration by means of immunofluorescence and immuno-gold techniques that new MTs form during anaphase in cells of the blood-lily, growing out from the polar region, which is also drastically altered towards the centre of the cell; polymerization of new MTs is involved in the activity of the anaphase spindle. B. Nicklas (Duke University, Durham) used extremely fine glass needles to detach single chromosomes from the spindle. He calculated the forces needed to move chromosomes: the power output per cm^3 is one million times smaller in the spindle than in muscle.

The dynamics of MTs cannot easily be studied in the intact cell. Nonetheless, E. Salmon (University of North Carolina) used an elegant method to demonstrate that treadmilling (growth of microtubules by assembly on one end and slower disassembly on the opposite end) obviously does not occur within the cell. Fluorescein-labelled tubulin was microinjected into sea urchin eggs and PtK₁ cells before mitosis. During formation of the spindle the labelled tubulin is homogeneously incorporated into all MTs. By exactly localized photobleaching and measurement of the redistribution of labelled material within the spindle he showed that recovery is much faster (400–600 dimers per s) than expected from treadmilling. Since fluorescein is irreversibly bleached the reappearance of the labelled material can only be explained by an incorporation of tubulin subunits along the whole length of spindle MTs.

Kinetochore and poles are the most important parts of the spindle. The structure of polar organelles can vary widely between cell types, but they all function as the mitotic pole. Therefore, it becomes questionable whether centrioles, the main polar organelle in higher animals, are only inactive passengers. N. Paweletz (German Cancer Research Center, Heidelberg) demonstrated in sea urchin eggs that the

shape of the spindle and the arrangement of the chromosomes are not defined by the centrioles but by the cyclic behaviour of the centrosome represented by dense osmiophilic material in the central part of the aster. B. Brinkley (Baylor College of Medicine, Houston) used human autoantibodies of scleroderma patients directed against kinetochores and immunofluorescence to show that kinetochores are already present in interphase nuclei. The number of prekinetochores corresponds exactly to the chromosome number. The kinetochores undergo cyclic alterations, are replicated in a complex process during interphase and appear doubled during G₂.

S. Wick (Australian National University, Canberra), working with plant cells, and G. Schatten (Florida State University, Tallahassee), with sea urchin eggs, showed by immunofluorescence drastic changes in the distribution of MTs during the transition from premitotic stages to mitosis. Both translocation of MTs and depolymerization-repolymerization are responsible for the respective microtubular patterns. Schatten also showed that no contractile ring of microfilaments is present in the cleavage furrow in sea urchin eggs. Thus other mechanisms than contraction must work during cleavage.

For the first time it was generally accepted that as well as MTs, membranes form part of many types of spindle. They have an essential role in sequestering regulating ions (for example calcium) and they obviously can serve as mechanical support. Using fluorescent dyes, P. Hepler (University of Massachusetts) showed a decrease in membrane-bound calcium shortly before anaphase while the fluorescence of different voltage-sensitive dyes exhibited either a rapid increase or remained constant during the transition from metaphase to anaphase. This transition obviously is accompanied by drastic changes in the distribution of calcium and other ions; this can only be effected by membranes. In mammalian cells, vesicles, mainly derived from the disintegrated Golgi apparatus and cisternae of the endoplasmic reticulum–nuclear envelope complex, are translocated into the spindle by means of MTs (Paweletz), while the sea urchin spindle is characterized by large amounts of vesicles which are involved in the regulation of the formation of the mitotic apparatus (P. Harris, Max Planck Institute for Biophysical Chemistry, Göttingen). Antibodies against the calcium transport enzymes of muscle cells inhibit the calcium-sequestering activities of vesicles isolated from the mitotic apparatus of sea urchin eggs. Anti-calcium transport enzyme IgGs can be used to identify the sites responsible for calcium transport (R. Silver, Chicago).

A very promising approach to the study of mitosis is the isolation and characterization of mutants varying from the wild type in spindle structure and function. B. Oakley (Ohio State University) and J. Gambino (Rutgers Medical School,

*The workshop, sponsored by the European Molecular Biology Organization and organized by J.S. Hyams, was held on 11–15 July.

Piscataway) have isolated and characterized tubulin mutants from *Aspergillus*, and identified multiple species of tubulins indicating multigenic control of tubulin production and MT polymerization.

Various types of MT can be distinguished by their sensitivity to nocodazole as well as to taxol (M. DeBrabander, Janssen Pharmaceutica Research Laboratories, Beerse). The pattern of assembly and disassembly varies strongly depending on the particular combination of drugs used, indicating different target sites for the two drugs. J. Hyams (University College) showed that methyl benzimidazol carbamate can be used as an inhibitor of MT polymerization in yeast. Thus, mitotic inhibitors for cell types which are less sensitive to the mitotic poisons used in higher plant and animal cells are now available.

W. Cande (University of California, Berkeley) has isolated and characterized a cytoplasmic dynein-like ATPase from sea urchin eggs which binds to different types of MT by bundling them in large parallel arrays, in which many cross-links can be found. The characteristics of the ATPase are consistent with its being a microtubule-associated protein (MAP) capable of cross-linking MTs in a Mg^{2+} - and ATP-dependent manner. By careful ultrastructural analyses and a computer-controlled program, C. Jensen (University of Auckland) demonstrated a highly ordered pattern of side arms and cross-bridges. Periodicities of 10–14, 20–24 and 48 nm are found along parallel MTs. Assuming side arms and cross-bridges represent MAPs, then either MAPs cannot be bound to all tubulin subunits in the MT; or different MAPs may have different binding sites, thus forming the different periodicities.

MAPs can show different patterns of distribution within different cells: whereas MAP2 exhibits a restricted cellular distribution in the brain, MAP1 is widely distributed and prominent in the mitotic spindle (R. Vallee, Worcester Foundation for Experimental Biology, Shrewsbury). Different types of MT can be characterized by their associated MAPs. It is possible that the diversity of MTs is due to their MAPs rather than to their tubulins.

Novel mechanisms for chromosome movement were not proposed. Looking back at all the data presented at the workshop, the attractive notion that one unique mechanism is responsible for the translocation of chromosomes seems no longer tenable. A large body of data has shown that sliding, assembly–disassembly and lateral interaction or zipping (to mention only the three main hypotheses) are all feasible. Clearly a combination of different mechanisms could be responsible for the chromosome transport. The workshop has also shown that we may also have to take into consideration mechanisms which have not yet been discussed at all. □

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Geophysics

Magma oceanography and the early evolution of the Earth

from Richard W. Carlson

A RECENT paper by Anne Hofmeister in *Journal of Geophysical Research*¹ presents a model for the evolution of a terrestrial 'magma ocean' created by heat from the impact of accreting planetesimals. Magma ocean models were applied to the Moon almost immediately after the first samples were returned by Apollo 11 (ref. 2) but it is only very recently that similar models have been seriously discussed for the early Earth.

During a planet's formation a large fraction of its energy is transformed to heat in the interior. The heat is lost by solid-state convection but, depending on how efficiently this takes place, additional energy received from the accretion of planetesimals may be sufficient to cause substantial melting. Given an adequate amount of melting, magma will coalesce to form a 'magma ocean' which, in the extreme case, could be of global scale and several hundred kilometres deep. As the magma ocean cools and crystallizes it can produce dramatic physical and chemical effects that will control the future dynamic evolution of the planet.

If the Moon did in fact undergo a magma ocean episode, then the Earth, because of its larger size and hence greater accretional energy, should have melted to an even greater extent. Understanding how a terrestrial magma ocean would behave is complicated by the fact that the phase relationships for the crystallization of silicate magmas at the higher pressures present in the Earth are not available. This limits modelling of a terrestrial magma ocean to a perhaps unrealistically shallow depth of about 120 km. Nevertheless, Hofmeister's work draws some interesting conclusions regarding the minimum size (laterally global), the time required for crystallization (< 10,000 yr; a factor of several hundred less than required for the Earth to form from accreting planetesimals) and the crystallization sequence of a 120-km-deep terrestrial magma ocean.

To a first approximation, the crystallization sequence of a terrestrial magma ocean that is initially similar in composition to the bulk Earth will resemble that believed to have occurred on the Moon. Put simply, the crystallization produces a layer cake of relatively dense Mg- and Fe-rich minerals (olivine, pyroxene) overlain by a thick crust of low-density plagioclase (a Ca, Al silicate). Plagioclase, being less dense than the magma it crystallizes from, will actually float to the surface of the ocean. These olivine- and plagioclase-rich layers will be convectively stable both with respect to each other and the underlying

mantle. In contrast, if the initial composition of the ocean is more similar to a partial melt of the mantle containing more Ca and Al than the bulk Earth, the increased pressure gradient of the Earth will allow the early and prolonged crystallization of the dense Mg, Fe, Al silicate, garnet. A major effect of garnet crystallization is that the deep garnet-bearing layers will be denser than the underlying solid mantle and will sink back into it. In this case, only a 30-km-thick plagioclase-rich crust will remain as evidence of a magma ocean episode.

In neither of the terrestrial magma ocean models¹ does this crust actually come close to matching the composition of the crust preserved in the oldest sections of the continents. This failure of a simple shallow magma ocean model to match the geological evidence is compounded further because, unlike rocks on the Moon, rocks of the Earth's crust lack the chemical and isotopic signatures expected to result from an early global molten episode.

While the visible geological evidence for a terrestrial magma ocean is lacking, or at least not obvious, the physical reality of a very hot early Earth is difficult to avoid. Whether this implies that the Earth managed to rid itself of its initial heat without undergoing extensive melting is uncertain. Other possibilities include erasure of the evidence for a magma ocean episode by convective mixing of the crust and mantle over Earth history, or that different and unexpected fractionation paths were caused by the crystallization of unusual high-pressure phases from a deep ocean. Another intriguing paper³ notes that because silicate liquids are more compressible than solids, there may be a depth in the mantle, estimated to be about 150 km, where liquids become denser than solids. In this event the terrestrial magma ocean would be confined to great depth and its subsequent evolution would be more difficult to predict.

Whatever the ultimate answer, attempts to understand the evolution of the Earth starting from a given set of initial physical conditions provide a welcome and much needed approach to understanding the early evolution of the solid Earth. □

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The new yeast genetics

Kevin Struhl

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Gene cloning and yeast DNA transformation techniques have greatly enhanced the power of classical yeast genetics. It is now possible to isolate any classically defined gene, to alter the yeast genome at will by replacing normal chromosomal sequences with mutated derivatives produced in vitro, and to create DNA molecules that behave as autonomous replicons or minichromosomes. These unique features of the new yeast genetics have been used to study many problems in eukaryotic molecular biology.

IN the past seven years, the genetics of the yeast *Saccharomyces cerevisiae* has been transformed from a classical field to a molecular one. The distinction does not concern the rationale for using genetic analysis to study biological problems, but rather procedural matters which nevertheless have profoundly influenced our knowledge of yeast cells. The manipulations of classical genetics involve the intact organisms. Mutations are identified by selecting for organisms having new properties and then characterized by analysing the progeny of sexual matings. In contrast, the distinguishing feature of the new yeast genetics is that the manipulations are performed directly on the genetic material itself. A region of the yeast genome is isolated by molecular cloning and mutations are generated by enzymatic or chemical treatment of the DNA. The phenotypic consequences of these mutations are then assessed on introduction of the mutant DNAs back into yeast cells.

The revolution in yeast genetics quickly followed the advent of recombinant DNA technology. Classically defined yeast genes were isolated as cloned DNA by virtue of their ability to be functionally expressed in *Escherichia coli* such that they complemented defects in a specific bacterial gene¹⁻³. Then, such cloned DNA segments were introduced into yeast cells, where they caused heritable changes; specifically they complemented defects in the analogous yeast genes⁴. The combined effect of these experiments and subsequent developments is the ability to manipulate the yeast genome by using artificial vectors of genetic information, previously impossible due to the lack of naturally occurring viruses that infect yeast. Of particular importance is the fact that cloned DNAs can be introduced exactly at their normal chromosomal locations or as part of artificially constructed chromosomes that behave strikingly like real ones. These unique features make the sophistication of yeast genetics considerably greater than that of other eukaryotic organisms, and in fact, the equal of prokaryotic organisms such as *E. coli*. Indeed, almost all the techniques of the new yeast genetics are analogous to those that have been used in *E. coli* for many years.

It is important to stress that although yeast genetics has been 'prokaryoticized', the organism itself is a eukaryote. The genetic material is confined to the nucleus in the form of chromatin, and it is organized on many chromosomes, each of which possess a centromere, two telomeres, and multiple sites of DNA replication. The transcription and translation processes have characteristic eukaryotic features (for example, three RNA polymerases, 5' capped-3' poly(A) mRNA which sometimes arises from spliced initial transcripts, no operons, translation beginning from the first AUG codon). Yeast cells contain distinctly eukaryotic proteins (histones, actin, tubulin, peptide hormones) as well as macromolecular assemblies or organelles that resemble those of higher eukaryotes, for example a nuclear membrane, mitochondria, 80S ribosomes, rough endoplasmic reticulum, Golgi apparatus and lysosomes. The biology of yeast involves the fusion of haploid germ cells of opposite mating

type to form diploid zygotes that, in appropriate conditions, undergo a fairly typical meiosis. It is a particular advantage that both haploid and diploid cells can be propagated vegetatively in defined medium. As such, they represent a form of cell culture in which the karyotype and other characteristics are maintained through many generations with sufficient fidelity to reproduce sexually. Thus, yeast is useful for studying many basic questions in eukaryotic biology, including gene expression, DNA replication, recombination, transposition, chromosome segregation, chromatin structure, secretion, the cell cycle, and the control of cell type (for general reviews see refs 5-12).

Here I shall first describe the principles of yeast molecular genetics with particular emphasis on those aspects that offer unique approaches to biological problems; then I will discuss how the methodology has contributed to our present knowledge of genes and chromosomes. Given the wide range of subjects being studied and the increasing population of yeast workers (since 1975, it has doubled approximately every 2 years), it is obviously impossible to review all recent developments.

Transformation and its genetic consequences

The transformation event is defined operationally as the selected and genotypic change of a yeast cell dependent on a particular DNA molecule. Experimentally, DNA containing a wild-type marker gene (M^+) is introduced into a strain that harbours a mutant copy (m^-) and M^+ transformants are selected from the original population. Typically, the m^- strain lacks an enzyme necessary for growth in certain conditions (for example, in medium lacking a particular amino acid), and the M^+ DNA encodes the enzyme. In essentially all experiments, M^+ transforming DNA molecules also contain sequences that permit replication in *E. coli* cells (as plasmids or phages) as well as genetic markers that allow for selection in *E. coli*. For reasons of technical ease, the DNA is usually isolated from *E. coli* cells.

In the standard transformation protocol⁴, spheroplasts from an m^- strain are prepared by enzymatically digesting the outer cell wall. These spheroplasts are incubated with M^+ DNA in the presence of polyethylene glycol and calcium chloride, then plated in selective conditions that also allow regeneration of the cell wall.

What happens to DNA molecules on introduction into yeast cells? The answer depends on the particular DNA sequences present on the transforming DNA (Fig. 1). Different modes of yeast transformation have been described^{4,13-16}; their properties are listed in Table 1 and are summarized below. Taken together, they constitute the basis for the new genetics.

Although most DNAs are unable to replicate autonomously in yeast cells, molecules containing segments of yeast DNA can be propagated if they recombine with homologous sequences in the host genome^{4,14}. A molecule containing two separate segments of yeast DNA can integrate at either genomic site. The transformants usually contain one copy of the transforming DNA per cell although multiple copies can integrate in tandem.

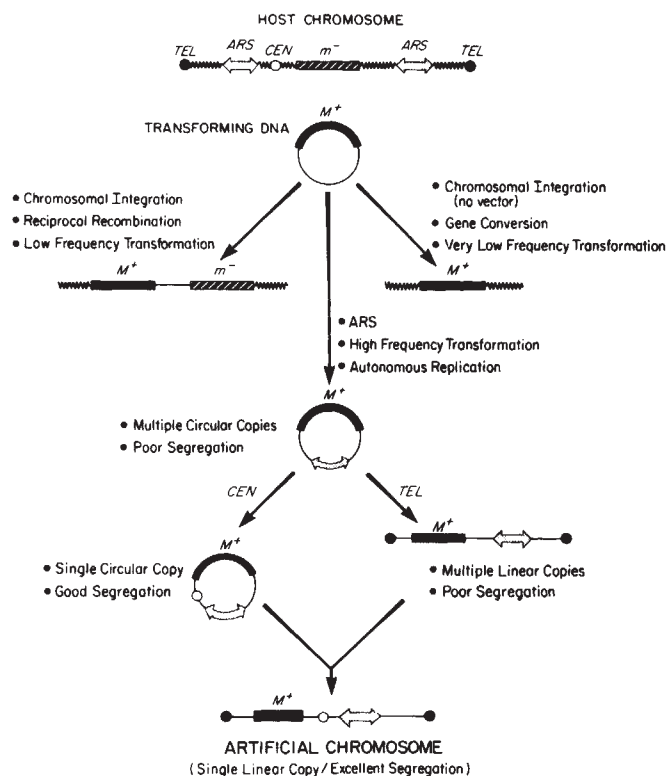


Fig. 1 Modes of yeast transformation/construction of artificial chromosomes. The top line represents a typical host chromosome with the following genetic elements: telomere (TEL, ●); centromere (CEN, ○); multiple replication origins (ARS, double-headed arrow); mutant sequences corresponding to the vector gene (m^- , striped bar); and other sequences (wavy line). The circular transforming DNA beneath the host chromosome consists of an M^+ marker (solid bar) and vector sequences (solid line). When introduced into yeast cells, this molecule integrates into the chromosome either by reciprocal recombination or by gene conversion (only the chromosomal region near the m locus is shown). Addition of cloned ARS, CEN or TEL elements produces molecules with various properties; when combined, they result in an artificially created chromosome (bottom of figure). See text for details.

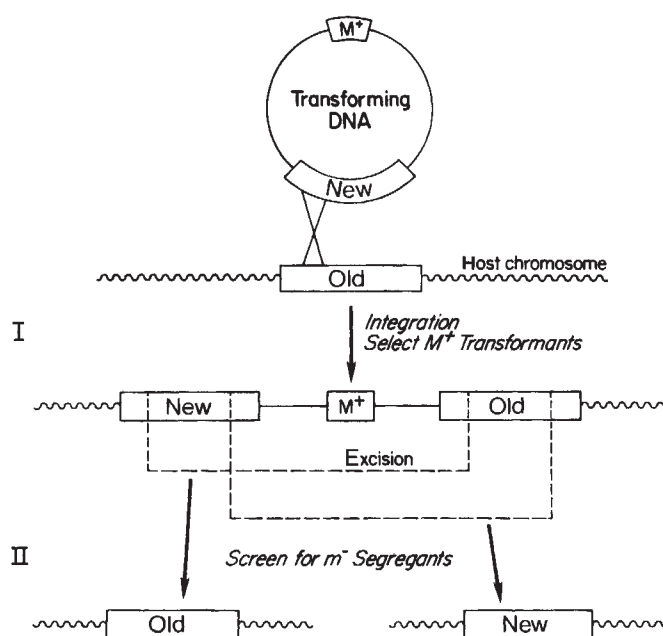


Fig. 2 Gene replacement is accomplished in two steps: integration then excision. The top part of the figure shows an homologous recombination event between a circular transforming DNA molecule and the normal linear chromosome (wavy line). The transforming DNA contains the M^+ vector marker and a 'new' cloned allele, while the original chromosome contains the 'old' allele. Integration results in a chromosome containing both alleles separated by the M^+ vector (middle of figure). The desired integration event can be obtained in several ways. Integrating vectors are generally used although these are inefficient because of their low transformation frequency and because integration can also occur at the normal chromosomal site of the M^+ marker³⁰. As described in the text, appropriate cleavage of the transforming DNA increases this transformation frequency and directs the site of integration^{17,107}; however, problems can arise because of multiple integration events. Alternatively, replicating vectors can be used to increase the transformation frequency and integration events identified by selecting for mitotically stable colonies¹⁹. The lower part of the figure shows the excision step. Two classes of segregants are obtained depending on whether the cross-over occurs to the right or to the left of the cloned mutant allele. m^- Segregants are generally identified by replica plating following growth in non-selective medium³⁰. The low frequency of excision (see Table 1) can be improved by selectively killing the M^+ population by inositol-less death¹²⁰. The two classes of segregants can be distinguished by virtue of phenotype or DNA structure. A method that allows for the direct selection of gene replacement events has been described recently³⁴. Using standard methods in a *cyh2'* (cycloheximide-resistant) strain, the wild-type *CYH2* gene¹²¹ was placed in the middle of the *his3* gene at the normal *his3* locus. The resulting strain is sensitive to cycloheximide because drug sensitivity (*CHY2*²) is dominant to resistance (*cyh2'*). Thus, gene replacement at the *his3* locus can be achieved by selecting for segregants that are resistant to cycloheximide.

Generally, the integrated DNA is transmitted for many generations; occasionally, however, it is excised by homologous recombination (Fig. 2). In some cases, transformants do not contain any copies of the transforming DNA⁴; that is, the original m^- allele is replaced by the M^+ copy from the transforming DNA. These are formally equivalent to two recombination events within the homologous sequences, but they probably arise by gene conversion. Because mitotic recombination is a relatively rare event, transformation via chromosomal integration occurs at low frequency (~1–10 colonies per microgram of transforming DNA).

Certain DNA sequences allow molecules to replicate autonomously in yeast cells and consequently to transform yeast at high frequency (1,000 to 10,000 colonies per microgram of DNA). These autonomously replicating sequences (*ars*) are derived from the endogenous yeast plasmid^{13,14,17} or from yeast chromosomal DNA^{14,18,19}. While *ars*-containing molecules replicate efficiently, they do not segregate properly during mitosis or meiosis. Although they are present on average in multiple copies (from 3 to 30 per cell depending on the molecule), many cells do not have any^{14,19}. However, autonomously replicating DNA molecules can be faithfully transmitted from mother to daughter cells if they contain yeast centromeric sequences (CEN) in addition to *ars* elements^{15,20}. Furthermore, such molecules are stably maintained at one copy per cell and they usually segregate properly during meiosis. Thus, they behave as circular minichromosomes.

When linear molecules are introduced into yeast cells, they either recombine with the genome or circularize and subsequently replicate. However, molecules can replicate in the linear form if, in addition to an *ars* element, they possess specialized end sequences such as those normally found at the ends of the linear *Tetrahymena* ribosomal DNA palindrome or at the telomeres of yeast chromosomes¹⁶.

Isolation of structural genes

The ability of yeast replicating vectors to transform both yeast and *E. coli* at high frequency and therefore to be readily interchanged between these two organisms makes it possible to clone essentially any gene for which phenotypically distinct mutations

Table 1 Properties of yeast transformation

	Chromosomal integration	Gene conversion	Episomal replicator	Chromosomal replicator	Mini-chromosome	Linear DNAs
Vector	I	I	E	R	C	L
Transformation frequency	10	1	10,000	10,000	10,000	10,000
Autonomous replication	None	None	Circular	Circular	Circular	Linear
Copies per cell	1	1	5–40	3–30	1	5–30
Vector sequences	Yes	No	Yes	Yes	Yes	Yes
Integration frequency	1	1	Variable	10 ⁻⁵	10 ⁻⁷	NT
Required elements	Yeast DNA	Yeast DNA	2 μ ARS	ARS	ARS CEN	ARS TEL
Mitotic loss	0.1%	0	30%	30%	1%	30%
Meiotic loss	1–10%	0	90%	90%	30%	90%

Each column represents a particular mode of yeast transformation. To use their distinct properties, yeast shuttle vectors were developed; for simplicity they are categorized as I, E, R, C and L. It should be noted that these distinctions, being somewhat arbitrary, break down in complex situations. The transformation frequency is measured in colonies per μ g of transforming DNA. In cases involving high rates of mitotic loss, the number of copies per cell represents an average. Except for I vectors, the integration frequency is calculated as events per generation; for I vectors, the frequency of 1 indicates that all transformation events require integration. (Chromosomal integration of E vectors is discussed in refs 14, 94 and 95.) Mitotic loss is measured on a per generation basis. Meiotic loss is determined by tetrad analysis and thus the values also reflect mitotic instability. CEN, centromeric DNA; TEL, telomeric DNA; NT, not tested.

are available. In other eukaryotic organisms, it is usually difficult to isolate a gene by virtue of its mutant phenotypes, and many interesting genes are defined solely in this manner. It has been estimated²¹ that over 100 previously defined yeast genes have been isolated by these methods (some early examples may be found in refs 14, 22–27).

The essence of the method is that defects caused by particular mutations can be 'cured' by introducing the cloned wild-type gene. Such genetic complementation is applicable for recessive mutations, by far the majority class. In practice, DNA segments representing the entire wild-type yeast genome are cloned into yeast replicating vectors, introduced into *E. coli*, and propagated *en masse*. The resulting hybrid DNA molecules are isolated and then introduced into an appropriate yeast strain—for example, one that fails to grow at high temperature due to a defective protein. Simultaneous selection for transformants that contain the vector marker and that grow at high temperature presumptively identifies the gene of interest. For more subtle phenotypes, transformed cells are first selected by virtue of the vector marker and then screened by whatever method serves to distinguish the phenotype of the recessive allele from its wild-type counterpart. Having obtained the desired complementation event, DNA is prepared from the relevant yeast transformant and introduced back into *E. coli*. The specific hybrid DNA when re-isolated from *E. coli* should complement the recessive mutation and hence should contain the cloned gene of interest. Given a reasonably complete collection of hybrid molecules, the entire procedure requires ~1–10 μ g of transforming DNA and takes about 2 or 3 weeks.

It is important to note that isolating DNA sequences by this procedure does not constitute proof that the desired gene has been cloned. Such proof may be established either by demonstrating that the identical DNA fragment from a mutant strain (isolated by nucleic acid hybridization to the presumptive cloned gene) is unable to complement the recessive mutation³, or by introducing the cloned DNA segment into the genome by homologous recombination and genetically mapping the integrated copy⁴.

To isolate the gene corresponding to a dominant mutation, the procedure is reversed. The hybrid pool is constructed by using genomic DNA from the strain containing the dominant mutation, and it is then introduced into a strain that contains the wild-type version. Transformants receiving the dominant allele are distinguished from the original host.

In some cases, genes can be cloned by indirect complementation (suppression). For example, the prediction that overproduction of the α mating type pheromone could suppress the phenotype of mutations in another gene (*bar1*) was used successfully to isolate the pheromone gene on a multicopy replicating vector²⁸.

Gene replacement

A critical feature of the new genetics is the ability to replace normal yeast chromosomal sequences with cloned DNAs. Given the awesome array of enzymatic and chemical tricks for mutating DNA, the yeast genome can be altered at will.

The gene replacement procedure (sometimes called transplacement) is analogous to an old *E. coli* technique, homogenization²⁹, in that it depends on homologous recombination between transforming DNA and the host genome^{4,14}. A simple scheme, taken from the first example³⁰, is shown in Fig. 2. An M^+ integrating vector containing a new cloned allele is integrated into the genome of an m^- strain at the chromosomal site specified by the original allele. In the relevant transformant, the new (transforming) and the old (host) alleles are separated by the M^+ vector sequence. Then, strains in which the integrated DNA has been excised are identified by screening for m^- segregants. Two classes of segregants are observed, both of which lack all vector sequences. One contains the original allele, while the other, more interesting, class represents replacement of the original host allele by the cloned one.

This two-step procedure is generally applicable, because its only requirements are that homologous recombination can occur on both sides of the lesion in the transforming DNA and that the transforming allele can be distinguished from the host allele in some manner. Because the desired recombination events are selected by unrelated genetic markers, these genomic changes can be made even if they produce subtle or no discernible phenotypes. Furthermore, by using diploid strains, it is possible to perform gene replacement on one chromosome while the essential function is supplied by the wild-type version on the homologue. By observing the properties of haploid cells that arise from such diploid strains, on meiosis, it has been proven that histones, β -tubulin and actin are essential for viability, and temperature-sensitive (*ts*) alleles have been created for some of them (refs 31–33, and subsequent work). A recent modification of this method facilitates gene replacement³⁴: instead of screening for rare segregants and then distinguishing between the two classes, a scheme has been devised that allows for the direct and independent selection of gene replacement events at a given locus (see Fig. 2 legend).

In comparing the molecular genetics with the classical variety, two additional points are worth noting. First, mutagenesis of DNA *in vitro* confines the sequence alteration to a small target. Previously, many mutations have been induced by harsh treatments such as X rays, UV light or chemicals. Because the entire organism is subjected to these mutagens, the strains that arise frequently contain multiple mutations. To isolate a desired mutation away from the others requires many backcrosses, a tedious exercise, and in addition it is difficult to know whether such

secondary mutations are present. Second, strain constructions can be performed without mating. As a result, it is easy to generate sets of isogenic strains that differ only at defined loci. This is in marked contrast to progeny derived from sexual crosses which have 50% of each parental genome. Also, constructions involving strains having mating or sporulation defects or with non-mendelian factors (the endogenous yeast plasmid, mitochondria or killer RNAs) do not constitute a problem.

Gene regulation

Although prokaryotic promoter and regulatory sequences were defined with classically isolated mutations, similar attempts in yeast have proved unsuccessful. In contrast, analyses of mutations produced by *in vitro* methods have enhanced our understanding considerably.

Given that yeast genes, unlike many *E. coli* counterparts, are not clustered in single transcription units (operons), it is difficult to distinguish undesired mutations in the structural gene from those affecting either its expression or regulation. The vast majority of mutations map in the structural gene simply because it is much larger than the control region. However, by *in vitro* manipulations, it is easy to alter DNA sequences in a highly localized manner. Another consideration that exacerbates the problem is that point mutations (by far the predominant class produced *in vivo*) frequently eliminate protein function, whereas their effects on promoter efficiency are less marked; thus many mutations 'escape the genetic screen'. On the other hand, the recent advances have depended on deletion, substitution and insertion mutations, all of which have more drastic effects on gene expression.

Furthermore, *in vitro* methods lend themselves to systematic changes in DNA sequence, in marked contrast to *in vivo* methods which rely on providence. Because test tube mutations can be obtained without regard to phenotype, mutations can be created without the inherent bias of a particular genetic selection or screen. Of particular benefit is the ability to produce mutations that behave indistinguishably from the wild-type gene. These are invaluable for determining which parts of the genetic material are not important for a given function; such information is precluded by classical genetic approaches. When analysed in conjunction with mutations that do cause functional changes, a much clearer view of a eukaryotic control region emerges.

Having obtained a set of *in vitro*-generated mutations, the next step is to assay their phenotype. The principle here is similar to experiments in which mutant DNAs are assessed following microinjection into frog oocytes or following transformation into mammalian cells. The difference between the new yeast genetics and these other approaches is that the DNAs are introduced back into the intact organism from which they were derived. Moreover, as DNAs are reintroduced in defined ways with respect to copy number and chromosomal location, the end results of these manipulations are equivalent to real genetics. These properties have some important consequences in studies of gene regulation. First, mutant genes are assayed in conditions in which all normal forms of regulation are occurring. Second, by using appropriate yeast strains, it is possible to determine how individual proteins (which are altered or removed by mutation) influence the expression of a given gene. Third, the absolute levels of products specified by mutant genes are directly comparable with their wild-type counterparts and with the levels of other genes. In other systems, levels of gene expression can be determined only relative to an arbitrarily defined DNA molecule.

Given the versatility of yeast transformation, mutant phenotypes can be analysed in several ways. The gene replacement procedure discussed previously constitutes the best possible *in vivo* assay for mutations produced *in vitro* and it has been used for studies of the histidine biosynthetic genes *his3* and *his4* (refs 35–37). Another approach—that used for the cytochrome *c* (*cyc1*)^{38,39}, alcohol dehydrogenase (*adr1*)⁴⁰, and mating type genes⁴¹—is to clone mutant DNAs into a yeast replicating vector and then select for transformants by using

the vector marker. Once again, the phenotype is determined independently of the transformation event itself. Although the autonomously replicating molecules are not identical to real yeast chromosomes, this procedure has several advantages compared with gene replacement. It is considerably simpler than gene replacement because: (1) replicating vectors transform yeast cells at high frequency; (2) the desired strains are obtained in a single step; and (3) complications due to recombination with the host genome are avoided. Furthermore, with the appropriate vector choice, it is possible to introduce the mutated derivatives in multiple or single copies per cell. Multiple-copy vectors allow for gene dosage studies that are difficult to perform by classical genetics. In either case, the resulting strains contain both the cloned mutant allele and the original host allele. By altering the host allele such that its gene product is distinguishable from the incoming allele (say, by a small deletion internally within the structural gene), such strains provide an excellent basis for *cis-trans* tests, analogous to *E. coli* strains containing F' factors or specialized transducing phages⁴².

Gene fusion, an approach used for nearly 20 yr to study prokaryotic gene regulation⁴³, has been adapted to yeast cells. Generally, the regulatory region under study is fused to a structural gene which can be assayed easily for genetic and biochemical function, for example the *E. coli* β -galactosidase or the herpes thymidine kinase genes^{44–46}. More importantly, complicated regulatory circuits can be dissected into individual components because the gene product that is assayed does not affect the regulation being analysed. For example, fusions of the (*cyc1*) regulatory region to the β -galactosidase structural sequences indicate that haem has an important role in gene control³⁹. By standard methods, this would have been difficult to elucidate because haem is also necessary for cytochrome *c* activity and presumably for its intracellular stability.

Even with the differences in approaches and genes, several general conclusions have emerged. A feature of yeast RNA polymerase II promoters is that DNA sequences located relatively far from the site of transcriptional initiation are critical for function^{35,37–40}. The actual distance ranges between 120 and 350 base pairs (bp) depending on the gene. Yeast promoters, like those of higher eukaryotes, also contain TATA box sequences and several studies have indicated their importance in gene expression^{35,38–40,47}. Further analysis suggests that for a given gene, the upstream promoter element can be moved with respect to the TATA box and mRNA start point with little phenotypic consequence^{39,47}. These properties differ radically from those of a prokaryotic promoter (reviewed in refs 48, 49), but they resemble those of viral enhancer sequences^{50–52}. That the upstream sequences confer their effects over a long and variable distance has suggested to many that this may involve chromatin structure. The gene replacement procedure has been used to examine the chromatin structure of *his3* mutant genes in their normal chromosomal location; the upstream element is necessary for preferential cleavage of the TATA box by micrococcal nuclease and the effect is not due simply to transcription of the gene⁵³.

In regard to proper control of expression in response to specific physiological cues, many genes have been shown to be regulated at the level of transcription, and several regulatory sites have been defined. Mutant studies of *his3* and *his4* have identified a particular sequence that is essential for proper regulation, but which is not critical for expression *per se*^{36,37}. This short sequence is repeated several times at the 5' ends of these and other genes controlled by the state of amino acid biosynthesis, for example *trp5* and *his1*^{54,55}. For genes controlled by the source of carbon in the medium (*adr1*, *cyc1*, *gal1*), the regulatory sites differ in sequence. However, all are located far upstream from the structural gene and they are critical for expression in addition to regulation^{38,40,56}. Chromatin studies of the *adr1* regulatory site indicate that the structure, as tested by DNaseI hypersensitivity, correlates with its function⁵⁷. By all these criteria, these regulatory sites resemble enhancer sequences which are effective only in certain cell lines⁵⁸. Regula-

tion of the mating type genes occurs by a completely different mechanism. Classical genetic experiments strongly suggested that the expressed copy at the mating type locus was similar to the silent copies located elsewhere⁷. Analysis of the cloned genes^{59,60} indicates that they are identical, thus leading to the idea that an unusual, long-range position effect is involved in regulating gene expression. Deletion analysis defines two regulatory sequences, each located ~1,000 bp past the 3' end of the mating type genes and only at the chromosomal sites specified by the silent copies⁴¹. These sites, in combination with regulatory genes defined previously⁶¹⁻⁶³, affect chromatin structure in terms of nuclease sensitivity and supercoiling such that gene expression from the silent copies is repressed^{41,64}.

In addition to regulatory sites, the control of gene expression also involves special regulatory proteins, most of which have been identified by classically isolated mutations. By the methods described earlier, genes encoding these presumptive regulatory proteins have been cloned, thus permitting new genetic studies^{59,60,65-68}. Of more importance, the cloned genes should prove invaluable in identifying, possibly overproducing, and hopefully purifying the gene products. Some of these, when overproduced, may be detrimental for cell growth. A potential way to avoid this problem is to fuse the structural gene of interest to an easily manipulated regulatory region, for example that specifying galactose control⁵⁶. Thus, by using unrelated physiological means (growth on galactose in this case), synthesis of a desired gene product could be induced just before collecting the cells. Thus, in principle, it should be possible in the future to correlate biochemical and genetic functions of regulatory proteins and their target sites.

A recent application of the new genetics has led to intriguing results concerning the splicing of intervening sequences from mRNA precursors. Using native genes or fusions in which β -galactosidase activity depends on accurate splicing, a conserved sequence has been found within the excised region which appears necessary for the processing reaction^{69,70}. The key sequence resembles that of U1 RNA, a small nuclear transcript found in higher eukaryotes that is believed to be important in splicing⁷¹. Thus, the proposed structure between U1 RNA and the splice junction in the precursor^{72,73} may be achieved in yeast by an intramolecular equivalent.

Chromosomes

Eukaryotic genomes encode other essential genetic information besides structural genes. Yeast transformation provides functional assays for isolating some of these elements. Presumptive chromosomal DNA replication origins allow hybrid molecules to transform yeast at high frequency, functional centromeres cause replicating molecules to segregate properly through mitosis and meiosis, and telomeres permit the replication of linear DNAs. Therefore, these previously elusive genetic elements can now be treated just like structural genes.

DNA replication: The issue of whether eukaryotic chromosomes contain specific origins of DNA replication has provoked interest and controversy^{74,75}. The ability of *ars* elements to permit autonomous replication of hybrid molecules strongly suggests that they serve as chromosomal origins; several observations support this view. The number of *ars* elements calculated by genetic experiments^{76,77} agrees well with the number of replication units as determined by fibre autoradiography⁷⁸. Regions of the yeast genome encoding a given *ars* element are replicated at characteristic times during the S phase of the cell cycle, and this specificity is maintained on autonomously replicating molecules⁷⁹. Finally, *in vitro* replication systems initiate DNA synthesis at *ars* elements⁸⁰⁻⁸², and these have been used to purify the protein specified by the *cdc8* gene^{83,84}.

ars elements contain conserved features within an extremely A+T-rich region⁸⁵. Their precise organization throughout the genome is not critical for viability as they may be inserted in new locations or even deleted from their normal one¹⁹. In two cases, it seems that these presumptive chromosomal origins are used to regulate certain genes. The histone H2b gene is normally

transcribed at a particular time during the cell cycle⁸⁶. By deletion analysis of appropriate gene fusions, the sequence responsible for this control maps far past the 3' end of the gene and coincides with an *ars* element⁸⁷. In the second case, the regulatory sites that control the position effect on the mating-type genes (see previous section) both coincide with *ars* elements^{41,85}. Such observations suggest that regulation of certain genes may be due to structural changes that arise in conditions when DNA synthesis is initiated from the relevant *ars* element.

Yeast transformation techniques have been invaluable for studies on the endogenous yeast plasmid because the naturally occurring molecule confers no detectable phenotype on its host. The plasmid replicon may be analogous to chromosomal ones in that the same genes are important for replication⁸⁸ and because each plasmid DNA molecule duplicates only once per cell cycle⁸⁹. By using various hybrid constructs⁹⁰, the yeast plasmid *ars* element has been mapped to the origin of DNA replication determined by electron microscopic techniques⁹¹, and genes responsible for copy number control and site-specific recombination within the plasmid have been identified^{90,92}. Although the yeast plasmid does not normally become integrated into the genome⁹³, hybrid molecules containing chromosomal sequences may do so by homologous recombination^{14,94,95}. Because the resultant chromosomes are frequently lost entirely, it is possible by using appropriately marked diploid strains to determine which chromosome contains the plasmid sequences⁹⁶. Thus, yeast DNA segments cloned in plasmid vectors can be mapped independently of their phenotype. In fact, many genes are more easily mapped by cloning them and using this chromosome loss method than they are by classical tetrad analysis.

Chromosome segregation: It has long been assumed that centromeres and telomeres have critical roles in ensuring that each daughter cell receives the proper chromosomal complement. The properties of cloned centromeric and telomeric sequences provide direct evidence for some of these roles and they have been used to define the important functional aspects. Recently, artificial linear minichromosomes have been constructed by combining *ars* elements, centromeric and telomeric DNA sequences in the same molecule (ref. 97; see Fig. 2). Their stability, though impressive, is still considerably less than for real chromosomes; this may be due to their small size^{97,98}.

Centromeric sequences are probably the primary determinant of proper chromosome segregation during mitotic and meiotic growth because autonomously replicating molecules containing a single *CEN* sequence segregate properly^{15,20}. However, when molecules containing two *CEN* sequences are introduced into yeast, the resulting colonies are heterogeneous for a mixed and continuously changing population of rearranged molecules⁹⁹. Such behaviour is reminiscent of 'breakage/fusion/bridge' cycles in maize¹⁰⁰, and it may result from mechanical breakage of individual DNA molecules attempting to segregate simultaneously to opposite poles. Given that centromeres in higher organisms are cytologically visible, it is perhaps surprising that yeast *CEN* function is confined to a very small region of DNA (<500 bp). Centromeres III and XI each possess three distinct sequence elements that are positioned in an almost identical spatial arrangement within the functional region¹⁰¹⁻¹⁰³. Centromeric sequences do not seem to be part of normal nucleosomal arrays *in vitro*¹⁰⁴; it has been suggested that this results from their interaction with the spindle apparatus. In this regard, a protein fraction that binds specifically to centromeric DNA may prove informative¹⁰³.

Studies of telomeric sequences indicate that they are necessary and sufficient for propagating linear molecules, and that they occur at the ends of most if not all yeast chromosomes¹⁶. Formation of the hairpin structures at chromosome ends depends specifically on telomeric sequences; artificially constructed hairpins are not sufficient to constitute functional telomeres¹⁰⁵. The fact that the ends of the *Tetrahymena* ribosomal DNA palindrome maintain their unusual gapped structure

at ends of replicating linear molecules in yeast suggests that telomere function is strongly conserved evolutionarily between kingdoms^{105,106}.

Preliminary studies using diploid strains containing two homologous, but genetically distinguishable minichromosomes have addressed a long-standing problem in genetics and physical chemistry—how do the duplex DNAs of homologous chromosomes pair during meiosis. It seems that circular and linear minichromosomes segregate preferentially to opposite poles, but that they segregate randomly with respect to the real chromosomes that contain the identical centromeres^{15,97}. This suggests that pairing may result from homology *per se* and not from a centromere and/or telomere organizing structure. In a more direct test of this hypothesis, the centromere of chromosome III was replaced by the centromere of chromosome XI; this novel chromosome pairs only with the native third chromosome, and it is equally stable during mitosis¹²².

Recombination: The frequency of transformation by non-replicating molecules can be increased dramatically by cleaving the DNA at sites that are homologous to yeast chromosomal DNA^{15,107}. The DNA ends are highly recombinogenic, presumably because they represent an intermediate in the normal process of repairing double stranded breaks¹⁰⁷. These events occur equally efficiently even when linear DNA substrates contain double-stranded gaps with respect to the native chromosome; furthermore, the gapped region on the transforming DNA is correctly repaired from the host copy. This reaction depends on expected recombination genes and it is associated with gene conversion and reciprocal recombination events near the site of the double-stranded break^{107,108}. The results agree well with experiments that demonstrate the formation of double-stranded breaks during the transportation event that results in yeast mating type interconversion¹⁰⁹. These considerations have led to the proposal that gene conversion in *S. cerevisiae* may be initiated by double-stranded breaks and subsequent DNA repair¹¹⁰. This model is an alternative to classical models that postulate invasion of duplex DNA by single-stranded DNA^{111–113}. Distinguishing between these models will probably depend on biochemical experiments¹¹⁰; in this regard, a recently developed *in vitro* recombination system may prove useful¹¹⁴.

Other experiments that take advantage of the ability to place homologous sequences at different chromosomal locations, have revealed additional features of gene conversion^{115,116}. First, it has been shown that recombination can occur between homologous sequences even if they are located on separate chromosomes. In the examples involving gene conversion

between different copies of the yeast transposable element *Ty1*, gene expression is frequently affected (ref. 116; for a recent review of the properties of this transposable element see ref. 11). This property has been used to generate specific reciprocal translocations¹¹⁷. Second, and more surprisingly, interchromosomal gene conversion occurs at the normal frequency even if the recombination breakpoints within homologous regions are separated by a large substitution of non-homologous DNA¹¹⁵.

The fate of classical yeast genetics

The invention of the automobile quickly relegated transportation by horse and buggy to a sentimental pastime. However, a similar fate is unlikely to be in store for classical yeast genetics. The major reason for this is that the new methods, while providing powerful tools for analysing previously defined genes and DNA sequences, are less useful for probing the unknown. Before genes can be cloned and manipulated, they must be identified. With the rare exceptions of genes that are known by virtue of their gene products, most are initially defined by mutations that confer particular phenotypes. Such mutations are more likely to be uncovered by a broad mutagenesis and screening procedure. Moreover, it is certainly easier to 'produce phenotypes' by altering wild-type sequences than by cloning wild-type genes, and the initial characterization of phenotype is frequently the way in which new biological phenomena are discovered. For example, yeast mutants defective in various stages of secretion have been used to understand various biochemical aspects of the overall process^{118,119}. It is hard to see how such mutations could have been found with the new yeast genetics. Another general approach for identifying new genes relies on mutations that suppress the phenotypes of other mutations. Suppression is frequently indicative of interaction (direct or indirect) between the gene products defined by the original and the suppressor mutation. The most revealing interactions are usually not specifically predictable, thus making the general search mode a more useful one. In addition, it should be noted that powerful genetic selections (capable of detecting 1 variant per 10¹¹ cells) are available for yeast, and these are often required to isolate suppressor mutations.

Thus, the new yeast genetics does not simply replace the standard approach, but rather is integrated with it.

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ARTICLES

Structure of the cosmic microwave background

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We predict the structure expected in the cosmic microwave background over large angular scales in realistic anisotropic and inhomogeneous universes. In a homogeneous anisotropic universe we find that there exist two possibilities, a quadrupole or a hotspot pattern with and without dipole contributions. A hotspot allows a direct observational test of an open universe. We show how infinite wavelength inhomogeneous perturbations of the Friedman universe can be represented as ensembles of different homogeneous universes.

THE cosmic microwave background radiation has propagated towards us through space and time carrying with it a wealth of information about the geometrical structure of the Universe and the distribution of matter within it. Small deviations from the perfect homogeneity and isotropy of the idealized Friedman cosmological models will be manifested as an angular variation of the radiation temperature over the sky^{1–3}. At present, one positive claim^{4,5} and two strong upper limits^{6,7} exist for the magnitude of any quadrupole component in the angular temperature distribution, $T(\theta)$. The upper limits correspond to $\Delta T/T \leq 10^{-4}$ over $>10^\circ$ and are derived from 50% and 80% sky coverage respectively.

We aim to predict some general features of the universal microwave background radiation. We consider the temperature profiles obtained in representative anisotropic and inhomogeneous cosmological models and show that in general there can arise two distinct sky patterns.

We investigate the 'hotspot' phenomenon first noticed by Novikov⁸ and examine its effect in inhomogeneous cosmological models. An observation of this effect offers the possibility of determining the total density of the Universe without the uncer-

tainties of non-luminous matter, unknown elementary particles⁹ or the calibration of the cosmic distance scale. We provide a physical interpretation of the hotspot and delineate the set of cosmological models in which it can and cannot occur. We also show how the sky pattern can be calculated for inhomogeneous universes by a particular superposition of sky maps for different homogeneous universes.

Homogeneous quadrupoles

The simplest, astronomically relevant cosmological models with non-isothermal microwave background temperatures are the homogeneous and anisotropic universes. They are finite in number. Their spatial geometries are described by the classification of homogeneous spaces first performed by Bianchi (see ref. 10); the one exceptional homogeneous cosmology that does not fall within the Bianchi class is the Kantowski-Sachs closed universe^{11–13}.

The simplest of the Bianchi models is the type I axisymmetric case and the metric is¹⁴ (general relativistic conventions follow ref. 15):

$$ds^2 = dt^2 - X^2(t)\{dx^2 + dy^2\} - Y^2(t) dz^2 \quad (1)$$

This provides a concrete example of the typical quadrupole temperature pattern found also in Bianchi types VII₀, VIII and

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IX when they are close to the flat Friedman model ($\Omega_0 = 1$, where Ω_0 is the ratio of the present cosmic density to the critical density ($3H_0^2/8\pi G$)). Today, at time t_0 , the shear tensor arising from (1) is

$$\sigma_{\alpha\beta} = \pm \frac{\sigma_0}{\sqrt{3}} \text{diag}(1, 1, -2) \quad (2)$$

where σ_0 is an arbitrary constant giving the amplitude of the shear anisotropy.

The geodesic equations describing the motion of photons can be solved exactly in the space-time (1)¹⁶. When the present anisotropy level is low, as observational limits indicate, then $\sigma_0 \ll H_0$, where H_0 is the current Hubble expansion rate. The present temperature pattern on the sky is then found to be a pure quadrupole in observed angle θ with temperature variation:

$$\frac{\Delta T(\theta)}{T_0} = \frac{T(\theta) - T_0}{T_0} = \pm \frac{4\sigma_0}{3\sqrt{3}H_0} (1 + z_L)^{3/2} P_2(\cos \theta) \quad (3)$$

where P_2 is a Legendre polynomial, T_0 the mean background temperature and z_L the redshift at which the microwave background was last scattered, $z_L \sim 10^3$. When the model is not axisymmetric, the spherical harmonics $Y_{2m}(\theta, \phi)$ determine the angular dependence on the right-hand side of equation (3). The $(1 + z_L)^{3/2}$ gives the time evolution of the anisotropy (in this model, and others with isotropic three-curvature $\sigma \propto (1 + z)^3$ exactly and in any model close to the flat Friedman universe we will have approximately $H \propto t^{-1} \propto (1 + z)^{3/2}$). If we use the observational upper limit^{6,7} of $\Delta T/T_0 \leq 10^{-4}$ then, for $z_L = 10^3$, equation (3) yields an upper bound of 1.6×10^{-8} for σ_0/H_0 , (compare refs 17, 18).

As a second example, and one which illustrates the existence of pure quadrupole temperature profiles in open ($\Omega_0 < 1$) anisotropic universes, we take the homogeneous Kantowski-Sachs models where, for pressureless matter, we solve the Einstein equations for $X(t)$, $Y(t)$ where

$$ds^2 = dt^2 - X^2(t)[d\theta^2 + f(\theta)d\phi^2] - Y^2(t)dz^2 \quad (4)$$

with $f(\theta) = \sin^2 \theta$ in the closed model, $\sinh^2 \theta$ for the open (Bianchi III) model and $f(\theta) = 1$ for the flat (Bianchi I) model (1). The evolution of the shear in the open model containing dust differs slightly from that in equation (1) when $1 + z < \Omega_0^{-1}$ since, asymptotically, $Y(t) \sim t$ and $X(t) \sim \ln t$ for large times. The amplitude of $\Delta T/T_0$ is increased over that in (1) by a factor $\sim |\ln \Omega_0| \approx 4$.

In all solutions of the geodesic equations for (1) and (4) there is neither focusing nor antifocusing of geodesic separations, θ_L , at the last scattering redshift, z_L , relative to their present separation on the sky, θ_0 , so

$$\theta_0 = \theta_L \quad (5)$$

The microwave background patterns are purely quadrupole in both these models. No peculiar velocities are admitted by equations (4) and (1), so neither possess dipole components.

The most general anisotropic universe containing the $\Omega_0 = 1$ Friedman model is of type VII₀. This model can contain peculiar velocities and hence a dipole component in addition to a quadrupole similar to (3). Unlike metrics (1) and (4), type VII₀ also possesses anisotropic spatial three-curvature and this creates an evolution of σ/H for models close to isotropy of the form¹⁷

$$\frac{\sigma}{H} = C(1 + z)^{-1} + D(1 + z)^{3/2} \quad (6)$$

The decaying (D) mode is simply the type I behaviour associated with the isotropic portion of the spatial curvature. The growing (C) mode is driven by the curvature anisotropy and is not present in type I; although its presence does not alter the VII₀ sky pattern from a quadrupole form, it alters the dependence of $\Delta T/T_0$ on σ_0/H_0 and, as shown below, has important implications for the description of inhomogeneous models.

Hotspots

If an anisotropic universe is open ($\Omega_0 < 1$) with space negatively curved, then a new phenomenon can occur that was first recognized by Novikov⁸. Geodesics can be focused ($\theta_L \gg \theta_0$) into a microwave 'hotspot' on the celestial sphere. In effect, the quadrupole pattern discussed above will be squeezed into a small region of the sky $\sim \Omega_0$ radians in scale (the focusing is dictated by the radius of curvature, and hence by Ω_0). The negative curvature causes the divergence of nearby geodesics as we follow them backwards in time to the surface of last scattering at z_L .

The simplest homogeneous, anisotropic universe in which this phenomenon occurs is the axisymmetric Bianchi type V model⁸. When the fluid source is dust, the metric and energy-momentum tensors are

$$ds^2 = dt^2 - X^2(t) e^{2z\sqrt{1-\Omega_0}}(dx^2 + dy^2) - Y^2(t) dz^2 \quad (7)$$

$$T_{ik} = \rho u_i u_k$$

where ρ is the matter density and u_i the fluid four-velocity and coordinates have been chosen so that equation (7) obviously reduces to equation (1) as $\Omega_0 \rightarrow 1$. When $X = Y$ then (7) is the open Friedman model. The shear tensor of the metric (7) is the positive solution of (2), while the three-velocity vector has a single non-zero component and

$$u_a(z) = \left(0, 0, \pm \frac{2\sigma_0(1 - \Omega_0)^{1/2}(1 + z)}{\sqrt{3}H_0\Omega_0}\right) \quad (8)$$

Note, therefore, that when $X \neq Y$, $\rho \neq 0$ and $\Omega_0 < 1$ this model necessarily contains a dipole component to $T(\theta, \phi)$ unlike (1) and (4).

The microwave temperature pattern is given, for small anisotropies, by the approximate formula (note some corrections to an analogous formula in ref. 17)

$$\frac{\Delta T(\theta)}{T_0} = \pm \frac{2\sigma_0}{\sqrt{3}H_0} g(\theta_0) \quad (9)$$

where the geodesic equations give the following form for $g(\theta_0)$

$$g(\theta_0) = \Omega_0^{-1}(1 - \Omega_0)^{1/2}[(1 + z_L)\cos \theta_L - \cos \theta_0] + (1 - \Omega_0)^{3/2}\Omega_0^{-2} \int_{\tau_L}^{\tau_0} P_2(\cos \beta) \text{cosech}^4(\tau/2) d\tau \quad (10)$$

with

$$\tau_0 = ch^{-1}(2\Omega_0^{-1} - 1) \quad \text{and} \quad \tau_L = ch^{-1}\left[\frac{2(\Omega_0^{-1} - 1)}{1 + z_L} + 1\right] \quad (11)$$

and $\theta_L = \beta(\tau_L)$ where

$$\cos \beta(\tau) = \frac{1 - \tan^2(\theta_0/2)\exp[2(\tau_0 - \tau)]}{1 + \tan^2(\theta_0/2)\exp[2(\tau_0 - \tau)]} \quad (12)$$

The focusing of geodesics (concave lensing) predicted by the solution (9–12) can be seen by noting that the relation between θ_0 and θ_L is now

$$\tan\left(\frac{\theta_0}{2}\right) = \tan\left(\frac{\theta_L}{2}\right) \exp(\tau_L - \tau_0) \quad (13)$$

We see that $\theta_L > \theta_0$ if, and only if, $\Omega_0 < 1$; when $\Omega_0 \rightarrow 1$ then τ_0 and τ_L approach zero and there is no focusing.

It is useful to note that when $\Omega_0 \ll 1$ the last term of $g(\theta_0)$ in (10) given by the right-hand term is proportional to

$$P_2[\cos\{2 \tan^{-1}(2 \tan(\theta/2)\Omega_0^{-1})\}] \quad (14)$$

In practice, this formula is a good description for larger Ω_0 values also.

In Fig. 1a we display the form of the angular function $g(\theta)$ for type V universes having densities $\Omega_0 = 0.1, 0.3$ and 0.95 . We would expect the scale of the spot feature to be time-dependent. Figure 1b shows the time evolution in a plot of microwave pattern $g(\theta)$ against redshift. For large z the pattern

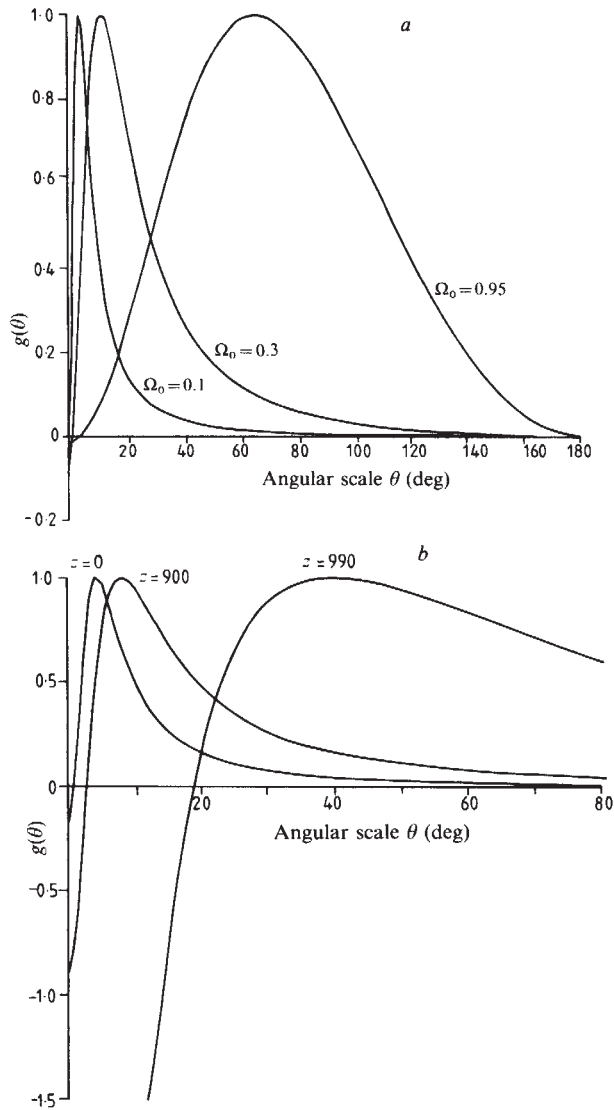


Fig. 1 *a*, The function of $g(\theta_0)$ in equation (10) in homogeneous universes of Bianchi type V with present Ω_0 values of 0.1, 0.3 and 0.95. Geodesics are focused into a region of small angular scale. *b*, The evolution of $g(\theta)$ with redshift for the type V universe. The form of $g(\theta)$ is plotted at redshifts 0, 900 and 990. At first the curvature has a minor role and the pattern is close to a pure dipole plus quadrupole. At redshifts less than $\Omega_0^{-1}-1$ the quadrupole pattern is focused into a region of small angular scale, so creating the hotspot feature.

is pure quadrupole, but it is focused into a region of decreasing angular scale as the redshift falls.

The most general anisotropic universe close to the open Friedman model is of Bianchi type VII_h. It is found to possess a hotspot sky^{19,30} of very similar angular form to that described by equations (9)–(14). However, unlike the type V model used as an example here, the VII_h model contains three-curvature anisotropy and the evolution of the shear in models close to the open Friedman universe is (at late times in the limit that the scale of curvature anisotropy, h , is infinite);

$$\frac{\sigma}{H} = \frac{C(1+3\Omega_0/2)}{(1+3\Omega_0/2+5\Omega_0z/2)} + \frac{D(1+z)^2}{(1+\Omega_0z)^{1/2}} + E(1+z)(1+\Omega_0z)^{1/2} \quad (15)$$

Here C , D and E are constants which determine the relative amplitudes. The decaying (D) mode is associated with the isotropic part of the curvature and is identical in form to the shear evolution in the type V model whereas the growing (C) mode is driven by the curvature anisotropy. (In fact, it can be

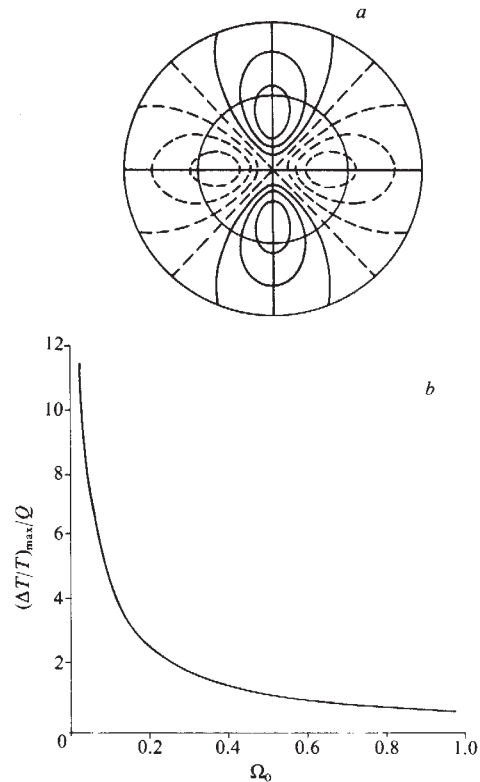


Fig. 2 *a*, A detailed map of the temperature profile $T(\theta, \phi)$ over a 10° region of sky centred on the hotspot in a non-axisymmetric version of the type V model in (7) with no velocity field (zero dipole contribution to $T(\theta, \phi)$). There is a $\cos(2\phi)$ azimuthal variation. The density is $\Omega_0 = 0.1$ and the temperature contours are 0.05K apart (solid lines denote $\Delta T > 0$, dashed lines $\Delta T < 0$). The model used to generate this picture has $\sigma_0/H_0 = 10^{-6}$ and hence a quadrupole temperature fluctuation $\sim 10^{-3}$. The fluctuation level in the vicinity of the hotspot can be very much larger (this figure supplied by R.J., P. Amsterdamski and M. Proszynski). *b*, The enhancement predicted at the spot temperature maximum compared with the quadrupole over the remainder of the sky outside the spot as a function of Ω_0 .

shown that all but a set of measure zero of VII_h universes have $\sigma/H \rightarrow C$ as $t \rightarrow \infty$ regardless of how close they are to isotropy, J.D.B. and S. Siklos, unpublished.) The remaining E -mode is decaying and generated by the curvature anisotropy.

The necessary condition for the presence of temperature hotspots is the simultaneous presence of cosmological anisotropy and low density ($\Omega_0 < 1$). The latter is not a sufficient condition, as the open Kantowski–Sachs model of equation (4) has $\Omega_0 < 1$ but no hotspot. We have investigated the structure of the homogeneous and anisotropic universes and find that hotspots occur in Bianchi types of Class B, except for the locally rotationally symmetric type III model (the open Kantowski–Sachs universe), but not in class A (for further details see J.D.B., R.J. and D.H.S., manuscript in preparation).

The appearance of hotspots is not linked to the relative generality of the models: Bianchi type VIII universes possess a quadrupole pattern but are more general open universes than those of type V. The failure to detect a characteristic hotspot in the microwave temperature distribution therefore would not tell us whether or not the universe was open. The positive detection of a hotspot would demonstrate unequivocally that the universe is open ($\Omega_0 < 1$) and its angular size would enable the value of Ω_0 to be determined precisely. (Its discovery would also rule out the inflationary universe scenario⁹, incidentally.) This value of Ω_0 would be the total density of the universe, including all non-luminous forms of matter.

The typical angular size of the hotspot is $\sim 80\Omega_0$ degrees and is considerably larger than the scale of features due to existing large-scale inhomogeneities like galaxy clusters which extend

only a few arc min. A spot feature would also be expected to occur in the cosmic X-ray background, although it would not be so strong as in the microwave region because of the comparatively recent origin of the X-ray background at z_L (X-ray) ~ 3 –5.

Figure 2a shows the detailed form of the temperature pattern expected in the vicinity of a type V hotspot, and Fig. 2b shows the relative intensity of the spot-peak against the background quadrupole as Ω_0 varies.

Inhomogeneities

So far we have described the principal characteristics of the microwave radiation distribution in anisotropic but spatially homogeneous universes. In reality, the Universe is inhomogeneous over large spatial scales and we might imagine that it can be described by a suitably weighted superposition of different homogeneous, anisotropic universe models. When the amplitude of the large scale inhomogeneities is small this idea can be made precise. The evolution of small pressureless density inhomogeneities in the flat Friedman universe is given by^{20,21}

$$\frac{\delta\rho}{\rho} = C(\mathbf{x})(1+z)^{-1} + D(\mathbf{x})(1+z)^{3/2} \quad (16)$$

These modes can be expressed as an ensemble of Bianchi type VII₀ models of differing density when $\delta\rho/\rho$ is less than unity. If Lifshitz's²⁰ expressions for the small metric perturbation, δg_{ik} , associated with these density inhomogeneities are examined in the zero wavenumber limit, $k \rightarrow 0$, then the δg_{ik} values have the form of zero-velocity Bianchi VII₀ models possessing an infinite value of the parameter x of ref. 17 (this x parameter is the length scale over which the principal axes of shear change their orientation in the model). From equation (6) we can see the explicit correspondence $\delta\rho/\rho \sim \sigma/H$ with the evolution of a single homogeneous model. The growing C -mode is intrinsic to the VII₀ evolution whereas the decaying D -mode is the result of an ensemble of type I solutions of the form (1) (although they will not in general be axisymmetric). In universes like our own which, if closed, are far from the expansion maximum, the result (16) applies also to closed ($\Omega_0 > 1$) models since type VII₀ approximates the general closed model^{17,19} of type IX at the relevant epochs when $p = 0$. The evolution of inhomogeneities in open universes close to Friedman is given by perturbation theory as^{20,21}

$$\frac{\delta\rho}{\rho} = \frac{C(\mathbf{x})(1+3\Omega_0/2)}{(1+\frac{3}{2}\Omega_0+\frac{5}{2}\Omega_0 z)} + E(\mathbf{x})(1+z)(1+\Omega_0 z)^{1/2} \quad (17)$$

This behaviour is equivalent to an ensemble of different Bianchi VII_h models. Again, the metric perturbation associated with these inhomogeneities tends, in the infinite wavelength limit ($\sqrt{k^2 - K} \rightarrow 0$, where $K < 0$ is the curvature parameter in the open model) to the form of the zero-velocity Bianchi VII_h model in the limit $h \rightarrow \infty$. At any moment of fixed time the metric of the VII_h model does not differ from the type V model with a peculiar velocity chosen to make it axisymmetric which we give in (7). An attempt to relate inhomogeneous cosmologies to homogeneous anisotropic ones via Bianchi I and V models alone has been made by Bisnovatyi-Kogan *et al.*²². In general the shear evolution of an inhomogeneous cosmological model is given by²³

$$\sigma_{ab} \sim (1+z)^3 [D_{ab}(\mathbf{x}) + \int_0^t A_{ab}(\mathbf{x}, t) [1+z(t)]^3 dt] \quad (18)$$

where A_{ab} is the three-curvature anisotropy, t the cosmic time and the matrix D_{ab} is time-independent. The connection between the C and E modes and A_{ab} can be seen explicitly.

We have calculated the variation of the root-mean-square (r.m.s.) temperature fluctuations, (or equivalently the angular correlation function $\omega(\theta)$) predicted by inhomogeneous flat and open models containing uncorrelated infinite-wavelength inhomogeneities (the closed models will give essentially the same results as the flat, $\Omega_0 = 1$, models since $\Omega_0 \approx 2$ in our Universe). The correspondence between the inhomogeneous models and

the homogeneous cases already enables us to conclude that there will exist just two types of microwave sky in inhomogeneous universes: either a superposition of quadrupoles or a superposition of hotspot skies; the presence or absence of dipole contributions will be determined by the presence or absence of peculiar velocities and our own intrinsic motion relative to the background radiation. This correspondence has another interesting consequence: limits¹⁷ on the present magnitude of σ_0/H_0 in the homogeneous Bianchi type VII₀ and VII_h universes will (to within small numerical factors) correspond to limits on the amplitude of C density perturbation modes in (16) and (17) today; whereas limits on σ_0/H_0 in types I and V give limits on the decaying E -mode in inhomogeneous models. We can therefore give limits on the amplitude of growing and decaying modes of shear in inhomogeneous models. It can be shown that the C and E mode fluctuations created in the microwave background are related, when the value σ_0/H_0 is fixed, by

$$\left(\frac{\Delta T}{T_0}\right)_{\text{slow decay}} = \left(\frac{\Delta T}{T_0}\right)_{\text{fast decay}} \frac{(1+\Omega_0 z_L)}{1+z_L} \quad (19)$$

$$\left(\frac{\Delta T}{T_0}\right)_{\text{grow}} = \left(\frac{\Delta T}{T_0}\right)_{\text{fast decay}} (1+z_L)^{-2} (1+\Omega_0 z_L)^{-1/2} \quad (20)$$

The value of $(\Delta T/T_0)$ for fast decay is given by the type V model, (9). If we assume that different quadrupoles and hotspots are uncorrelated (the Copernican principle) over scales larger than the horizon—this is quite reasonable since individual hotspots are outside each other's light cones at z_L —then we can predict the r.m.s. temperature fluctuations in the flat and open models.

Consider the sky pattern arising from a random superposition of quadrupoles. This will be the case in flat inhomogeneous models and also in a class of special open inhomogeneous models that resemble an ensemble of Kantowski-Sachs models of type (4) with $f(\theta) = \sinh \theta$. (Exact examples of the latter type are given by the inhomogeneous Szekeres models^{24,25} of Kantowski-Sachs type in which the expansion scale factors in (4) generalize to possess a dependence $Y(t, z, \theta, \phi)$ and $X(t)$. An analysis of the geodesic equations reveals that the Szekeres inhomogeneous models have quadrupole skies for all values of Ω_0). We consider the case of a single infinite-wavelength inhomogeneity mode. If N type VII₀ quadrupoles are randomly orientated, the resulting temperature anisotropy will be

$$\frac{\Delta T(\theta)}{T_0} = \sum_{r=1}^N \frac{\Delta T_r(\theta - \theta_r)}{T_0} \quad (21)$$

with each $\Delta T_r/T_0$ given by an expression of the form (3). The resulting correlation function

$$\omega(\theta) = \frac{\langle \Delta T(\alpha) \Delta T(\alpha + \theta) \rangle}{T_0^2} \quad (22)$$

is calculated to be

$$\omega(\theta) = \frac{32}{135} (1+z_L)^3 \frac{\langle \sigma_0^2 \rangle}{H_0^2} \cos 2\theta \quad (23)$$

where the r.m.s. shear induced by the inhomogeneities is

$$\langle \sigma_0^2 \rangle = \sum_{k=1}^N \langle \sigma_0^2 \rangle_k \quad (24)$$

The second type of sky pattern arises from a random superposition of hotspot skies and occurs only when $\Omega_0 < 1$. In this case the superposition of VII_h models will produce a sky similar to that generated by N hotspot skies of the type described by (8–13) orientated at random in the manner of (21) and the resulting form of $\omega(\theta)$ can be calculated numerically. Figure 3a shows the predicted forms for $\omega(\theta)$ for $\Omega_0 = 0.1, 0.3$ and 0.95 .

The predictions for the growing and decaying modes have the same variation of ω with θ but differ in amplitude because of the redshift variations. The $\Omega_0 = 0.95$ case is almost equal to the quadrupole pattern resulting from a superposition of pure quadrupoles predicted by (23) for $\Omega_0 = 1$. We see that the

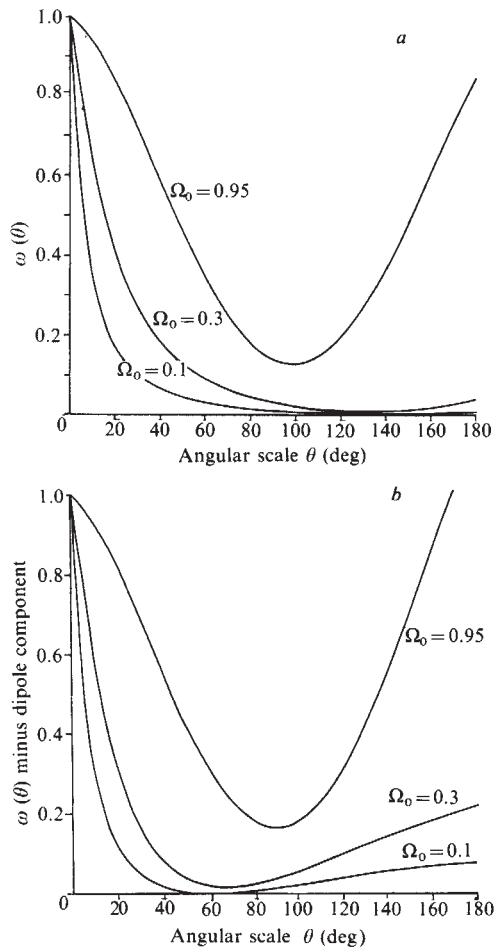


Fig. 3 *a*, Autocorrelation function $\omega(\theta)$, for inhomogeneous universes with $\Omega_0 = 0.1, 0.3$ and 0.95 , including dipole contribution. *b*, As *a* but with dipole variation subtracted.

variation of ω with θ is predicted to be a sensitive function of Ω_0 and future measurements of $\omega(\theta)$ should enable these predictions to limit the possible values of Ω_0 . To facilitate a direct comparison with observational data we follow the observers' practice⁴⁻⁶ and plot a reduced correlation function, $\varpi(\theta)$, remaining after the dipole component is subtracted from the total signal where $g(\theta)$ is taken from (10). The correlation function, $\varpi(\theta)$, after the dipole has been subtracted is

$$\varpi(\theta) = \omega(\theta) - \left[\int Y_{10}(\theta) \omega(\theta) d\Omega \right] Y_{10}(\theta) \quad (25)$$

and the multipole decomposition is defined by

$$T(\theta) \equiv T_0 \left(1 + \sum_{l,m} a_{lm} Y_{lm} \right) \quad (26)$$

$$|a_l|^2 = \sum_{m=-l}^{m=l} |a_{lm}|^2$$

the resulting forms of $\varpi(\theta)$ are shown in Fig. 3b.

It is also instructive to determine the decomposition of $|a_l|^2$ with l ; this is shown in Fig. 4. There is an interesting trend to this decomposition as Ω_0 falls. When $\Omega_0 = 1$, there is a pure quadrupole ($l = 2$) harmonic, but for $\Omega_0 < 1$ the hotspot superposition leads to a different prediction: a dipole harmonic appears, generated by the velocities of form (8) in the superposition of decaying mode fluctuations in (19) of the type produced by metric (7). The spots themselves create a build-up of power at l -number $l_* \sim \pi/\Omega_0$ dictated by the angular size of the individual hotspots.

As Ω_0 falls, the dipole remains constant but the relative importance of the quadrupole falls and the spot introduces

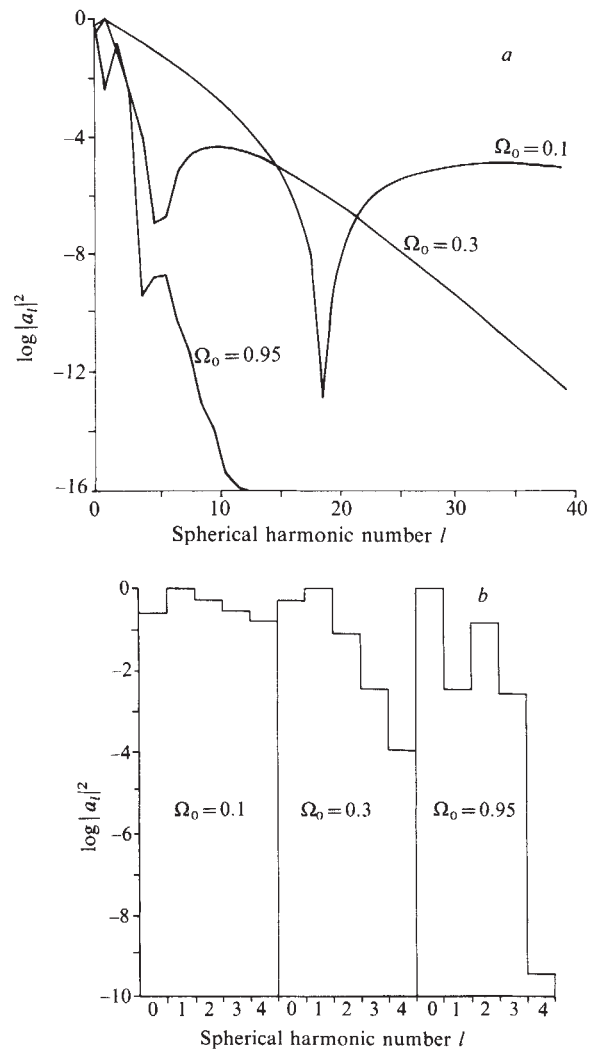


Fig. 4 *a*, Multipole analysis of the fluctuations in Fig. 3 for $\Omega_0 = 0.1, 0.3$ and 0.95 . The values of $|a_l|^2$ are scaled so that the largest value of $|a_l|^2$ is unity. *b*, Histograms of the data in *a* giving amplitudes of the first five l -poles for $\Omega_0 = 0.1, 0.3$ and 0.95 .

power at larger l numbers since the angular extent of individual spots decreases with decreasing Ω_0 . Similar trends can be observed in the result of Fabbri *et al.*²⁶ and Wilson²⁷ who both utilized the gravitational instability theory of Lifshitz²⁰ to calculate the temperature fluctuations produced in the microwave background by density inhomogeneities larger than the present horizon size. Their results are easily interpreted in the light of our analysis. The very high frequency component found at $l \gg 2$ by Fabbri *et al.*²⁶ in their inhomogeneous perturbations of open Friedman universes has a simple interpretation as the hotspot phenomenon. The decrease of the quadrupole to dipole ratio as Ω_0 falls can be seen in the results of Wilson²⁷, which were derived by different techniques to those used here—this did not allow him to give a physical explanation for the high-frequency tail at $l \gg 1$ when $\Omega_0 < 1$.

We have also calculated the maximum present amplitude of infinite wavelength inhomogeneity, $(\sigma_0^2)^{1/2}/H_0$, compatible with the present observational upper limits of $\omega(\theta) \leq 9 \times 10^{-10}$ for $\theta \geq 10^\circ$, under the assumption that the inhomogeneities are uncorrelated on wavelengths exceeding cH_0^{-1} . The limit is plotted as a function of Ω_0 in Fig. 5 for both the growing and decaying modes. The reason for including the decaying mode is that isothermal density perturbations contain only decaying modes today, as they do not generate significant spatial curvature inhomogeneity²⁸.

Adiabatic inhomogeneities will contain both modes but the growing mode will predominate today. As Ω_0 increases, the

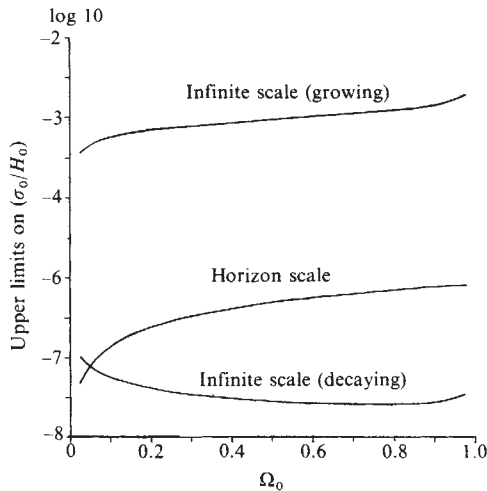


Fig. 5 The maximum amplitude of uncorrelated growing and decaying mode shear anisotropy of infinite wavelength compatible with the observation of the microwave background quadrupole fluctuation. Also shown is the shear expected from the observed inhomogeneity in the luminous matter distribution on scales $\leq cH_0^{-1}$. We take $1+z_L = 10^3$.

gravitational effect of the inhomogeneity increases and the bound on the maximum allowed amplitude tightens. These results generalize those of Grishchuk and Zeldovich²⁸, obtained for $\Omega_0=1$, to the case $\Omega_0 < 1$. For perspective we have also included in Fig. 5 the value of $\langle \sigma_0^2 \rangle^{1/2}/H_0$ that must exist on the scale cH_0^{-1} today due to the gravitational influence of the observed inhomogeneity in the luminous matter distribution of the Universe. If the amplitude of the mass inhomogeneity in the present horizon volume of the Universe is denoted by $\langle (\delta M/M)^2 \rangle_H^{1/2}$ then the tidal shear associated with it is calculated to be²¹

$$\frac{\langle \sigma_0^2 \rangle^{1/2}}{H_0} = \frac{\Omega_0^{0.6}}{\sqrt{3}} \left\langle \left(\frac{\delta M}{M} \right)^2 \right\rangle_H^{1/2} \quad (27)$$

This can be related to the observed two-point correlation function of galaxies, $\xi(r)$ since^{21,29}

$$\left\langle \left(\frac{\delta M}{M} \right)^2 \right\rangle_H = \frac{4\pi}{V_H(\Omega_0)} \int_0^{r_H} \xi(r) r^2 dr \approx \frac{4\pi J_3}{V_H(\Omega_0)} \quad (28)$$

where $V_H(\Omega_0)$ is the present horizon volume. The integral in (27) is well-approximated by Peebles' moment function J_3 when r_H is large (J_3 is the value of the integral in (28) when $r_H = \infty$). For the observed $\xi(r)$ we have²⁹ the estimate $J_3 = 10^3 h_0^{-3} \text{ Mpc}^3$ where h_0 is the Hubble constant in units of $100 \text{ km s}^{-1} \text{ Mpc}^{-1}$, and so (27) and (28) yield σ_0/H_0 versus Ω_0 which is also plotted in Fig. 5. (In models in which the large-scale structure of the Universe is generated by violent events on subgalactic and galactic scales after recombination, the referee points out that J_3 should be close to zero on scales exceeding $\sim 0.01 c t_0$ and

so the associated quadrupole distortion could be smaller by 10^2 – 10^3 .)

We can also compare our results with those of Peebles^{2,29} who calculated the integrated gravitational effect of inhomogeneities of all wavelengths rather than that created by a single eigenmode as did Wilson, Fabbri *et al.*²⁶ and ourselves. Peebles assumes that the inhomogeneities are random, $\langle \xi(r) \rangle = 0$, on scales exceeding some $r_* < cH_0^{-1}$. The density inhomogeneities have a power-law spectrum with $\delta\rho/\rho \propto M^{-n}$ with $n = 0.5$ for $r > r_*$. The results obtained for the r.m.s. temperature fluctuations are qualitatively similar to ours (see Fig. 3), but we note that the form found by Peebles for $\omega(\theta)$ when $\Omega_0 < 1$ is not a result of the small scale inhomogeneity where $\xi(r) \neq 0$, but rather is the result of the 'background' white noise with $\xi(r) = 0$ on scales with $r > r_*$.

When $\Omega_0 < 1$, the white noise on scales exceeding r_* gives rise to a random distribution of hotspots of the form described by (7)–(12) as in our Fig. 3. This explains the form of Peebles' results, with $\omega(\theta)$ more sharply peaked around $\theta = 0^\circ$ as Ω_0 decreases. There is one important difference between Peebles' calculations and our own: he has a dipole present in the $\Omega_0 = 1$ model which is possible because he includes finite wavelength inhomogeneities, while we do not. These short wavelength perturbations create a dominating dipole fluctuation through their peculiar velocities.

The quadrupole to dipole ratio a_2/a_1 created by inhomogeneities of wavenumber k in the flat model is proportional to kcH_0^{-1} , and this tends to zero in the limit $k \rightarrow 0$ we study. In this limit both the microwave radiation and the observer become freely expanding with the Hubble recession and their relative velocity tends to zero. We are not considering the dipole effects because it is impossible to determine whether a measured dipole fluctuation arises in this way or whether it is created by our own peculiar velocity (a point made also by Peebles²⁹).

Conclusions

The class of open universes in which the hotspot phenomenon occurs has thus been established. The discovery of a hotspot would provide definitive evidence for an open universe and allow a direct determination of Ω_0 to be made from the angular extent and intensity gradient in the vicinity of the hotspot. We find that it is possible to model inhomogeneous universes close to the Friedman model as superpositions of the most general homogeneous Bianchi models containing the Friedman models as isotropic special cases. This approach to calculating the behaviour of microwave photons in inhomogeneous models differs from that used previously and enables simple physical interpretations to be made of the expected temperature fluctuations.

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Mantle plume noble gas component in glassy basalts from Reykjanes Ridge

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Basalts from the Reykjanes Ridge contain noble gases delivered from the non-degassed lower mantle by the Iceland plume. These lower mantle gases are thought to be a mixture of planetary and solar components, as would be expected if the Earth accreted from fine silicate particles.

DEEP-SEA glassy basalts dredged from the Reykjanes Ridge have intermediate isotopic ratios of $^{87}\text{Sr}/^{86}\text{Sr}$ and $^{206}\text{Pb}/^{204}\text{Pb}$ between those from Iceland and mid-ocean ridge basalts (MORB), suggesting that their source may be a mixture of Icelandic mantle plume and MORB sources^{1,2}. On the basis of rare-earth element (REE) abundances, Schilling³ suggested that the Iceland mantle plume source may be undifferentiated and primordial. However, O'Nions *et al.*⁴ have shown that the $^{143}\text{Nd}/^{144}\text{Nd}$ ratio for the Iceland source is higher than chondrite meteorites, indicating the Iceland source must have suffered some preferential loss in light REE relative to chondrites. In contrast, the lead-isotope study of these samples suggests a mantle source having higher U/Pb and Th/Pb ratios than chondrites, estimated values for the bulk Earth, or the mantle source of MORB^{2,5}. This suggests the U/Pb and Th/Pb ratios for the Icelandic mantle source have been enriched, perhaps by removal of Pb to the core⁶. Finally, the $^3\text{He}/^4\text{He}$ ratio in these samples is higher than either MORB, seawater, or continental reservoir ratios, which suggests a source representative of a component having a primordial $^3\text{He}/^4\text{He}$ ratio^{7,8}.

Recent studies have shown that the $^{40}\text{Ar}/^{36}\text{Ar}$ and $^3\text{He}/^4\text{He}$ ratios of the large-ion lithophile (LIL)-depleted MORB source are more radiogenic (high $^{40}\text{Ar}/^{36}\text{Ar}$ and lower $^3\text{He}/^4\text{He}$) than samples from Hawaii or the samples from Reykjanes Ridge reported here⁷⁻¹². This observation has led to the suggestion that ^3He and ^{36}Ar were extensively degassed from the MORB source in the Archaean^{10,13}. The resultant enrichment of the K/ ^{36}Ar and U-Th/ ^3He ratios in the Archaean has led to high $^{40}\text{Ar}/^{36}\text{Ar}$ and low $^3\text{He}/^4\text{He}$ ratios in the modern MORB source by radiogenic production of ^{40}Ar from K, and ^4He from U and Th (refs 10, 13). On the other hand, basalts from Hawaii and the Reykjanes Ridge appear to be derived from a mantle source that has not been extensively depleted in ^{36}Ar or ^3He (refs 10-13). In fact, the $^{40}\text{Ar}/^{36}\text{Ar}$ ratio of most Hawaiian basalts and the Reykjanes Ridge basalts reported here are only slightly higher than the atmospheric ratio of 295. Four explanations of

the similarity of the $^{40}\text{Ar}/^{36}\text{Ar}$ ratio of non-degassed mantle and atmosphere have been proposed:

(1) The atmosphere has degassed from the mantle in recent times¹⁴.

(2) Atmospheric noble gases have cycled through the mantle¹⁵.

(3) The samples are contaminated by atmospheric Ar during or after emplacement on the seabed¹⁶.

(4) The atmosphere degassed solely from the upper mantle MORB source while leaving a remnant of noble gases in the non-degassed mantle. Subsequently, aside from production of ^{40}Ar from ^{40}K , both the non-degassed portions of the mantle and the atmosphere have retained their original inventories of Ar (refs 10, 13). The small differences in their ratios reflect the retention of some ^{40}Ar produced from decay of ^{40}K in the upper mantle and sialic crust without degassing to the atmosphere^{10,13}.

Explanation (1), that of late atmospheric degassing, does not account for the high $^{40}\text{Ar}/^{36}\text{Ar}$ ratios in MORB. Furthermore, numerous samples of mantle origin have $^{129}\text{Xe}/^{130}\text{Xe}$ ratios significantly higher than that of the atmosphere¹⁷⁻²². Since ^{129}Xe is produced by decay of ^{129}I with a half life of 17 Myr, the atmosphere and mantle must have been separated within a few hundred thousand years after the cessation of the nucleosynthetic production of ^{129}I in the solar nebulae^{17,19,21}.

Similar arguments rule out explanations (2) and (3), that is, mixing of atmospheric gas with either the mantle or eruptive silicate melts does not provide an explanation for the high $^{40}\text{Ar}/^{36}\text{Ar}$ ratio in the K-depleted MORB source. In addition mixing of atmospheric gas would probably eradicate the $^{129}\text{Xe}/^{130}\text{Xe}$ difference between the atmosphere and mantle. Finally, we provide evidence that the mantle source of Reykjanes Ridge basalts have $^3\text{He}/^4\text{He}$, $^4\text{He}/^{36}\text{Ar}$, $^3\text{He}/^{36}\text{Ar}$, $^{40}\text{Ar}/^{36}\text{Ar}$ and $^{20}\text{Ne}/^{36}\text{Ar}$ ratios different from those of the atmosphere. We, therefore, chose explanation (4), that of closed system evolution of a non-degassed portion of the mantle beneath Iceland coupled with extensive degassing of the MORB source

Table 1 Noble gas abundances in glassy basalts from the Reykjanes Ridge*

Sample No.	Description	^4He ($\times 10^{-7}$)	^3He (10^{-11})	^{20}Ne (10^{-10})	^{36}Ar (10^{-9})	^{40}Ar (10^{-7})	^{84}Kr (10^{-11})	^{132}Xe (10^{-12})
TR101-1D	Tholeiite at 62° 41' N; 1,800 m depth	4.78	0.82†	8.66	0.30	0.95	0.87	0.65
TR101-1D	Tholeiite at 62° 41' N; 1,800 m depth	4.82	—	12.83	0.43	1.30	2.09	2.29
TR101-2D	Tholeiite at 62° 30' N; 800 m depth	2.86	0.62†	6.72	0.25	1.09	0.37	0.27
TR101-27D	Tholeiite at 62° 46' N; 1,000 m depth	7.11	1.45†	8.21	0.38	1.28	1.66	1.14
TR101-27D	Tholeiite at 62° 46' N; 1,000 m depth	8.47	—	10.22	0.45	1.53	1.01	—

* Isotopic ratios of neon, krypton and xenon are indistinguishable from those of the atmosphere. Units, $\text{cm}^3 \text{g}^{-1}$.

† Analysed by R. Poreda⁷.

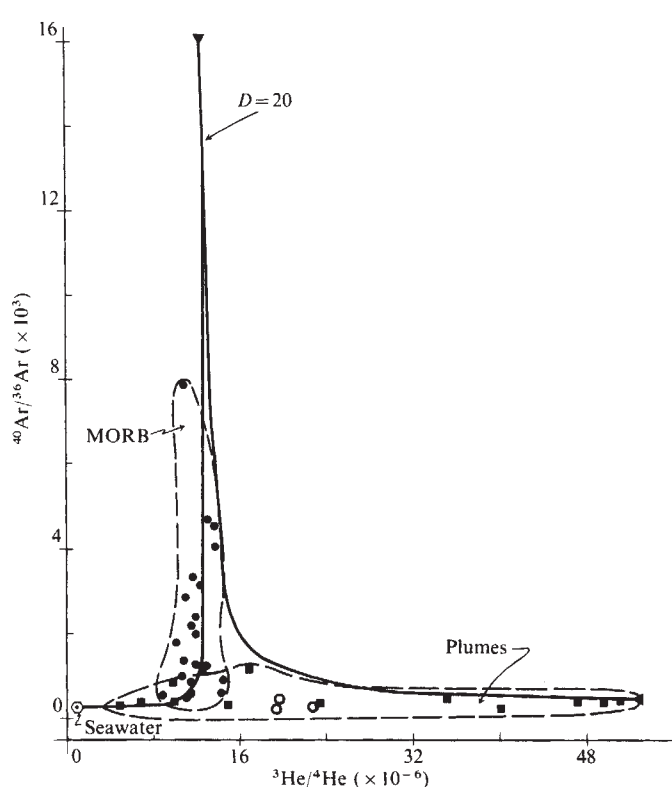


Fig. 1 The $^3\text{He}/^4\text{He}$ and $^{40}\text{Ar}/^{36}\text{Ar}$ ratios of Reykjanes basalts (○) are compared with other plume and MORB samples. The MORB samples (●) are from the Galapagos Rise and Mid-Atlantic Ridge 22–36° N (from refs 9, 11, 12, 24 and L.H. unpublished data). The plume field (■) is defined by samples from Hawaii (from refs 11, 12, and L.H. unpublished data). Mixing lines are from ref. 13. The curvature of the mixing line is a function of $D \equiv ^{36}\text{Ar}/^{3}\text{He}$ ratio for the non-degassed mantle divided by $^{36}\text{Ar}/^{3}\text{He}$ ratio for the degassed mantle. The high $^{40}\text{Ar}/^{36}\text{Ar}$ and low $^3\text{He}/^4\text{He}$ ratios of the MORB samples are attributed to Archaean degassing of ^{36}Ar and ^3He from the upper mantle. The U, Th and K contents of the upper mantle were depleted less than ^{36}Ar and ^3He , resulting in a build up of $^{40}\text{Ar}/^{36}\text{Ar}$ and a decrease of $^3\text{He}/^4\text{He}$ with time. The atmosphere has lost He relative to Ar and ^3He relative to ^4He from the upper atmosphere. Therefore, the atmosphere has a different $^3\text{He}/^4\text{He}$ signature than the deep mantle. On the other hand, both mantle sources for the plume source and atmosphere have approximated closed systems for Ar, so the difference in the $^{40}\text{Ar}/^{36}\text{Ar}$ ratio of the plume source and atmosphere is mainly the ^{40}Ar that has built up in the upper mantle and sialic crust from decay of K still in the upper mantle or transferred from the upper mantle to sialic crust.

to form the atmosphere. Below we present evidence to support the hypothesis that the Reykjanes Ridge glasses contain a noble gas component from a non-degassed mantle source. Later we compare the noble gas patterns in the Reykjanes Ridge basalts with those from planetary and solar sources.

Non-degassed mantle beneath Iceland

The evidence that the Reykjanes Ridge basalt samples contain a mantle plume noble gas component stems from their argon and helium isotopic composition. The rare gas compositions of three glassy basalts dredged by Schilling³ from a depth of 800–1,000 m on the Reykjanes Ridge are given in Table 1. A detailed account of sample preparation analysis and comparisons of duplicate runs with other laboratories is given elsewhere²³. Here we briefly state that the basaltic glass samples were broken into 1.84–6 mm fragments. In our laboratory large fragments are used in preference to fine powder to eliminate gas loss from vesicles during crushing. The glass fragments were ultrasonically cleaned in double distilled water to remove palagonite and other alteration products. A split of the clean glass fragments was sent to Scripps Institution of Oceanography and analysed for ^3He

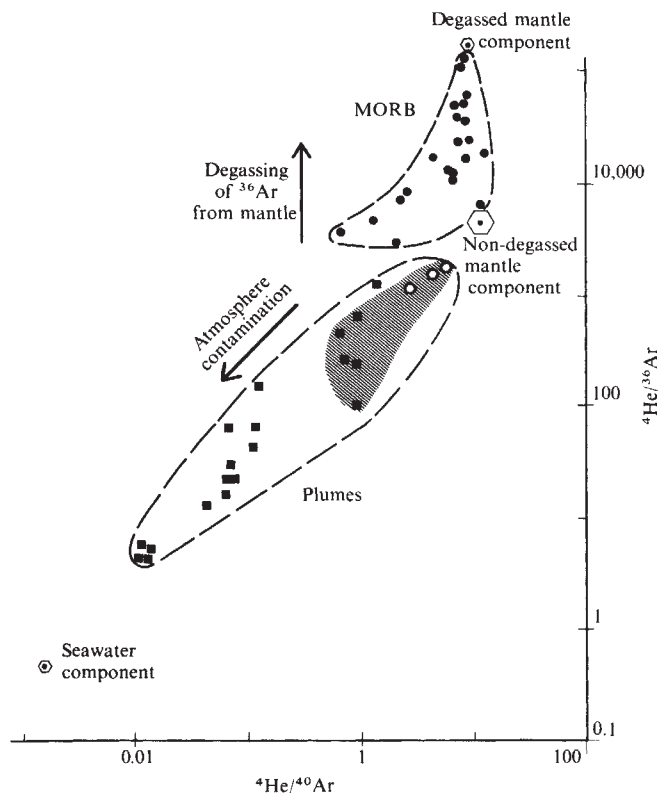


Fig. 2 The $^4\text{He}/^{40}\text{Ar}$ and $^4\text{He}/^{36}\text{Ar}$ ratios in Reykjanes Ridge basalts (○) are compared with those of other plume and MORB sources. The MORB field (●) is defined by data on samples from the Galapagos Rise and Mid-Atlantic Ridge 22–36° N (from refs 9, 11, 12, 24 and L.H. unpublished data). The plume field (■) is defined by samples from Hawaii (from refs 11, 12 and L.H. unpublished data). This plot suggests the noble gas composition of glassy submarine basalts can be explained by mixing of a seawater component, a degassed mantle component, and a non-degassed mantle component. These components are represented by the hexagons with dots. The $^4\text{He}/^{40}\text{Ar}$ ratio for the non-degassed mantle component was calculated from equations for closed system decay using a K/U ratio in the range $1\text{--}1.27 \times 10^4$ (from ref. 2) and a Th/U ratio in the range $1\text{--}3.7$ (from ref. 42). The closed system range for $^4\text{He}/^{36}\text{Ar}$ was derived by multiplying the $^4\text{He}/^{40}\text{Ar}$ ratio by the $^{40}\text{Ar}/^{36}\text{Ar}$ ratio of the lower mantle (300–500) which has been calculated by assuming both the lower mantle and the atmosphere are closed systems for Ar, and the difference between their $^{40}\text{Ar}/^{36}\text{Ar}$ ratios is equal to the amount of ^{40}Ar still in the degassed mantle and in the sialic crust (from ref. 13). The shaded area encompasses samples with $^3\text{He}/^4\text{He}$ ratios higher than the MORB average of 12.6×10^{-6} .

by R. Poreda. Details of the ^3He analysis are given elsewhere^{6,24}. Our glass splits were loaded in Pyrex furnaces and outgassed for 30 h at 200 °C. Stepwise heating experiments in our laboratory demonstrate this procedure eliminates atmospheric noble gas contamination. After determination of background, the sample was dropped into a clean degassed molybdenum crucible, and the sample fused. ^3He and ^{38}Ar spikes were introduced, and the gases were cleaned on titanium getters and introduced into a Reynolds-type mass spectrometer for analysis. The average background corrections are: 6% for ^4He , 42% for ^{20}Ne , 8% for ^{40}Ar , 18% for ^{36}Ar , 22% for ^{84}Kr , and 53% for ^{132}Xe . The (2σ) precision determined on duplicate runs in our laboratory is 18% for ^4He , 30% for ^{20}Ne , 9% for ^{36}Ar , 1% for ^{40}Ar , 16% for Kr, and 76% for Xe. In Fig. 1, the results for $^{40}\text{Ar}/^{36}\text{Ar}$ and $^3\text{He}/^4\text{He}$ ratios are compared with those of other oceanic basalts. This plot has been used to describe mixing of an atmospheric component, MORB component and plume component in the oceanic crust^{11–13}. The Reykjanes Ridge samples plot in the plume field but are offset towards the MORB field, suggesting a significant admixture of depleted upper mantle magma and/or atmospheric noble gases.

Table 2 Results of crushing experiment (mean grain diameter = 0.2 mm)

Sample no.	^4He ($\times 10^{-6}$)	^{20}Ne ($\times 10^{-10}$)	^{36}Ar ($\times 10^{-9}$)	^{40}Ar ($\times 10^{-7}$)	^{84}Kr ($\times 10^{-11}$)	^{132}Xe ($\times 10^{-12}$)
TR101-1D	0.06	3.80	0.30	0.93	1.77	3.49
TR101-2D	0.03	2.50	0.27	0.88	1.80	3.17
TR101-27D	0.27	6.04	0.36	1.21	1.92	2.36

Units, $\text{cm}^3 \text{g}^{-1}$.

The $^4\text{He}/^{40}\text{Ar}$ and $^4\text{He}/^{36}\text{Ar}$ ratios can be used to discriminate between atmospheric, MORB and plume components. The theoretical $^4\text{He}/^{40}\text{Ar}$ ratio for a closed system decay of U-Th and K of the mantle composition lies in the range 8–13 (refs 13, 25, 26). The theoretical $^{40}\text{Ar}/^{36}\text{Ar}$ ratio for non-degassed mantle is in the range 300–500 (refs 10, 13). The $^4\text{He}/^{36}\text{Ar}$ ratio for a hypothetical closed mantle can be calculated from the $^4\text{He}/^{40}\text{Ar}$ and $^{40}\text{Ar}/^{36}\text{Ar}$ ratios to be in the range $2.4\text{--}6.5 \times 10^3$. Figure 2 is a plot of $^4\text{He}/^{40}\text{Ar}$ against $^4\text{He}/^{36}\text{Ar}$ in which the Reykjanes Ridge samples are compared with other oceanic basalts as well as some ultramafic nodules and josephinite. We also show in Fig. 2 the most probable values for a MORB source, non-degassed mantle source, and the atmosphere. The Reykjanes Ridge basalts fall within the field of admixture between the MORB-source, the atmosphere and non-degassed closed system bulk Earth, but closest to the latter type of probable reservoirs.

Because significant amounts of He have been lost from the atmosphere²⁷, the $^4\text{He}/^{40}\text{Ar}$, $^4\text{He}/^{36}\text{Ar}$, and $^3\text{He}/^{36}\text{Ar}$ ratios of the atmosphere are up to 10^6 times lower than the same ratios in the Reykjanes Ridge samples. This observation rules out significant contamination of the Reykjanes Ridge samples by atmospheric argon. However, several samples from Hawaii form a distinct mixing trend towards atmospheric $^4\text{He}/^{40}\text{Ar}$ and $^4\text{He}/^{36}\text{Ar}$ values in Fig. 2, suggesting the Hawaii samples have been contaminated by atmospheric argon and helium to variable degrees. This is not surprising because some Hawaii basalts had to travel through an old, probably seawater hydrothermally altered crust–lithosphere slab and sediment blanket before erupting, whereas this is not the case for the zero age basalts of the actively spreading Reykjanes Ridge. Manuel and Sabu¹⁵ have postulated ^3He from a nonspecified portion of the mantle mixes with mantle contaminated by atmospheric argon and thereby account for the high $^3\text{He}/^4\text{He}$ and $^3\text{He}/^{36}\text{Ar}$ ratios while maintaining low-atmospheric $^{40}\text{Ar}/^{36}\text{Ar}$ ratios. The Manuel and Sabu model does not account for the high $^{40}\text{Ar}/^{36}\text{Ar}$ ratios in the K-depleted MORB source and faces the serious problem of eradicating the mantle $^{129}\text{Xe}/^{130}\text{Xe}$ anomaly by introduction of atmospheric Xe. In addition, it is unlikely that introduction of helium from another mantle source into non-degassed mantle beneath Iceland would result in the exact $^4\text{He}/^{40}\text{Ar}$ ratio predicted by closed system decay. Finally, the Reykjanes Ridge samples also have $^{20}\text{Ne}/^{36}\text{Ar}$ ratios significantly higher than that of the atmosphere.

Comparison with planetary and solar sources

Before discussing the broader implications of the rare gas data, it is necessary to outline briefly the systematics of the rare gas composition of the oceanic crust. Serious discrepancies have come to light from intra-laboratory comparisons of the rare gas concentrations of oceanic glassy basalts^{11,24}. Fine-grained samples with adsorbed gases that have not been degassed before fusion often show the effects of mixing of atmospheric gases. Furthermore, 10–85% of the helium content of glassy basalts is in the vesicles and is released during sample crushing⁹. In Table 2 and Fig. 3 we present the results of crushing experiments on the Reykjanes Ridge basalts. The finest grain samples have lost helium and neon relative to argon, probably because the light rare gases are partitioned into vesicles and are then preferentially released during crushing. On the other hand, krypton

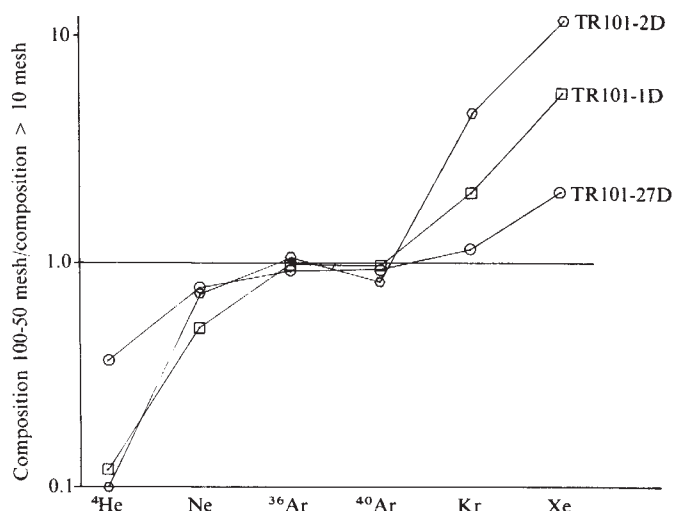


Fig. 3 Results of crushing and vesicle degassing experiment are displayed. The noble gas composition of Reykjanes Ridge samples ground to 100–50 mesh (mean grain size, 0.2 mm) is normalized to the composition for the greater than 10 mesh size (mean grain size ~ 2 mm).

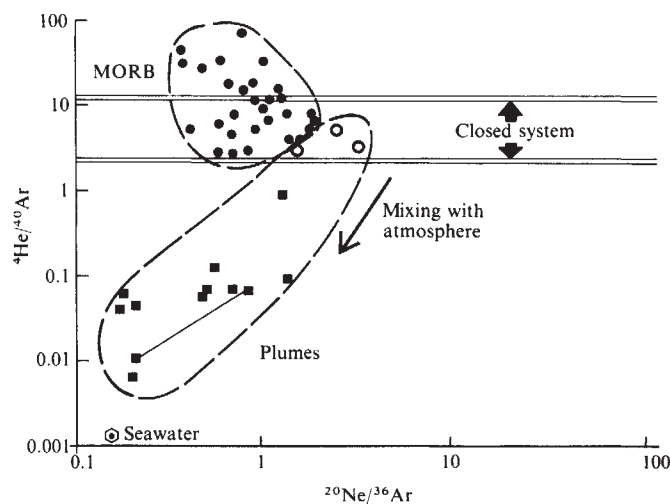


Fig. 4 The $^4\text{He}/^{40}\text{Ar}$ and $^{20}\text{Ne}/^{36}\text{Ar}$ ratios of Reykjanes Ridge glasses are compared with those of other plume and MORB glassy basalts. Data are from ref. 11, and L.H. unpublished data. The closed system range for the $^4\text{He}/^{40}\text{Ar}$ ratio is calculated from the U, Th and K concentration of oceanic basalts¹³. High values of the $^4\text{He}/^{40}\text{Ar}$ ratio may be the result of the higher solubility of He in silicate melts compared with Ar. There is no corresponding increase in the $^{20}\text{Ne}/^{36}\text{Ar}$ ratio with the trend noted for $^4\text{He}/^{40}\text{Ar}$, suggesting the high $^{20}\text{Ne}/^{36}\text{Ar}$ ratios are not a result of the higher solubility of ^{20}Ne relative to ^{36}Ar . The arrow connecting two plume samples represents duplicate analysis on a plume sample from the East Rift Zone of Kilauea (from ref. 11, and L.H. unpublished data). The difference probably reflects different amounts of outgassing of atmospheric contamination before extraction.

and xenon have been enriched in the fine-grained samples, probably a result of preferential adsorption of the heavy rare gases from the atmosphere on the finer grained material. A similar effect of adsorption of xenon has been observed on terrestrial shales and is thought to be responsible for the low Xe content in the Earth's atmosphere²⁸. We suggest that many discrepancies, particularly that of the $^{20}\text{Ne}/^{36}\text{Ar}$ ratio, can be traced to the use of different size fractions in the noble gas extraction process.

The effect of atmospheric mixing on the $^{20}\text{Ne}/^{36}\text{Ar}$ ratio is demonstrated in Fig. 4. The degassed and air-contaminated samples with low $^4\text{He}/^{40}\text{Ar}$ ratios also have low $^{20}\text{Ne}/^{36}\text{Ar}$ ratios as expected from post eruptive degassing and atmospheric contamination. The high $^{20}\text{Ne}/^{36}\text{Ar}$ ratios are similar to those

Table 3 Comparison of isotope ratios of Reykjanes glasses with seawater, cosmic and planetary abundances

Sample no.	$^3\text{He}/^{36}\text{Ar}$	$^{20}\text{Ne}/^{36}\text{Ar}$	$^{36}\text{Ar}/^{84}\text{Kr}$	$^{36}\text{Ar}/^{132}\text{Xe}$
TR101-1D	0.028	2.70	33	445
TR101-1D (repeat)	—	2.69	21	188
TR101-2D	0.025	2.48	66	897
TR101-27D	0.038	1.95	23	334
TR101-27D (repeat)	—	2.06	45	—
Seawater*	0.004×10^{-5}	0.15	24.5	320
Murray (including gas-rich separates)†‡	0.008–0.016	0.062–0.53	152–272	102–1237
Planetary†	0.009	0.28	80	96
Cosmic abundance§	1.56¶	22.4	5,200	10,400

* From ref. 11. † From ref. 38. ‡ From ref. 37. § From ref. 39.

|| $^3\text{He}/^4\text{He} = 1.43 \times 10^{-4}$ (ref. 40); ¶ $^3\text{He}/^4\text{He} = 1.8 \times 10^{-4}$ (ref. 41).

reported in other glassy oceanic basalts and are often attributed to the higher solubility of ^{20}Ne in silicate melts^{11,29–31}. However, ^4He is even more soluble than ^{20}Ne so the solubility effect should be even greater for the $^4\text{He}/^{40}\text{Ar}$ ratio than for $^{20}\text{Ne}/^{36}\text{Ar}$. As shown in Fig. 4, samples with $^4\text{He}/^{40}\text{Ar}$ ratios higher than those predicted for closed system decay do not have enhanced $^{20}\text{Ne}/^{36}\text{Ar}$ ratios. In addition the extremely good correlation of the $^{36}\text{Ar}/^{84}\text{Kr}$ ratio for all terrestrial materials has been suggested as evidence against mass fractionation due to solubility differences in silicate melts^{15,23}. Consequently, we are led to believe the noble gases in the Reykjanes Ridge glassy basalts may contain a relatively non-fractionated component of the deep mantle primordial rare gases.

We now turn to a brief comparison of the noble gas signature of the Reykjanes Ridge glasses with that of meteorites and solar wind (Table 3). The discovery of anomalies in the abundances of isotopes of several elements in meteorites suggests that the protoplanetary gas nebula was chemically heterogeneous. Manuel and Sabu¹⁵ propose that the primitive nebula contained several types of noble gases. Type Y gas contained only Ar, Kr and Xe and was dominant in regions of accretion of terrestrial planets. Type X gas contained all five noble gases, He, Ne, Ar, Kr and Xe and was concentrated in the outer regions of the primitive nebula. In addition protoplanetary debris was bombarded by noble gases from the solar wind having a signature now found on lunar surface fines and in some brecciated carbonaceous chondrites^{32–34}. The first suggestion that the Earth's interior may contain a remnant of the solar noble gases was based on the high $^{20}\text{Ne}/^{36}\text{Ar}$ ratio reported by several laboratories^{11,31,35}. Most recently, a $^3\text{He}/^4\text{He}$ ratio of 200×10^{-6} has been reported on a diamond from South Africa³⁶. This is considerably higher than the ratio of 143×10^{-6} measured in planetary type meteorites³⁷ and suggests some material in the Earth's mantle was irradiated by the solar wind.

As shown in Table 3 and Fig. 5, the $^3\text{He}/^{36}\text{Ar}$, $^{20}\text{Ne}/^{36}\text{Ar}$, and $^{36}\text{Ar}/^{132}\text{Xe}$ ratios of the Reykjanes glasses are less than solar ratios, but greater than the same ratios in the planetary patterns or gas-rich separates from chondrites. We therefore

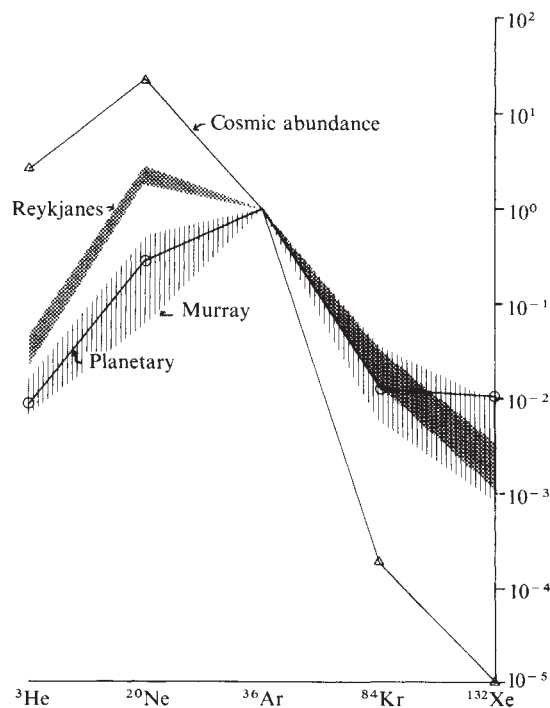


Fig. 5 Comparison of the noble gas abundance pattern measured for the Reykjanes glasses with the cosmic abundance patterns from ref. 39, the abundance pattern of noble gases in the gas-rich separates from the Murray carbonaceous chondrite from ref. 37 and the average planetary abundance patterns from ref. 38. This plot suggests the Earth's deep mantle noble gases could be a mixture of planetary and solar noble gases.

suggest the Earth's mantle contains two components: (1) solar gases rich in ^3He and ^{20}Ne that were implanted on fine-grained silicate dust during accretion of the Earth, and (2) ambient Earth orbit nebulae gases rich in ^{84}Kr and ^{132}Xe , scavenged, absorbed and occluded by the accreting Earth as it moved around the Sun. The Earth orbit noble gases may be similar in composition to the noble gases in the gas-rich carbon residues in carbonaceous chondrites³⁷.

Conclusions

We conclude first that the high $^3\text{He}/^4\text{He}$, $^3\text{He}/^{36}\text{Ar}$, $^4\text{He}/^{36}\text{Ar}$, $^4\text{He}/^{40}\text{Ar}$ and $^{20}\text{Ne}/^{36}\text{Ar}$ ratios measured in Reykjanes Ridge basaltic glasses rule out significant atmospheric mixing either in the mantle source or after emplacement on the seabed. Second, that the measured $^4\text{He}/^{40}\text{Ar}$ and $^4\text{He}/^{36}\text{Ar}$ ratios for the Reykjanes basalts are close to the value predicted by a closed system model in which the atmosphere degassed from the MORB mantle source leaving a remnant of primordial noble gases in the source of the Iceland mantle plume. Finally, the noble gas abundance patterns for these samples are also consistent with a non-degassed primordial component that is a mixture of planetary type noble gases trapped during condensation of protoplanetary particles and solar gases implanted during bombardment of the particles by the solar wind during Earth accretion. This work was funded by NSF grants OCE80-24285 and EAR82-06031.

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LETTERS TO NATURE

Broad emission lines of QSOs are consistent with rotating supermassive stars

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Quasars and Seyfert galaxies typically have optical and UV spectra characterized by a largely nonthermal continuum, narrow emission lines, and broad emission lines (full widths at zero intensity $\sim 10^4 \text{ km s}^{-1}$) observed only in permitted and intercombination transitions. The broad lines come from a small amount of dense, fast-moving gas, in a region $\sim 10^{18} \text{ cm}$ across near the continuum source¹. The origin, geometry, and dynamics of the broad line gas are uncertain. I suggest here that the basic properties of the broad emission-line profiles are consistent with emission from the surface of a rotating, supermassive star.

The broad-line intensity ratios can be understood in terms of optically thick clouds of solar composition photoionized by the UV and X-ray continuum^{1–3}. This requires gas densities $N \approx 10^{9.5} \text{ cm}^{-3}$ and an 'ionization parameter' $\Gamma_{\text{UV}} = \phi_{\text{UV}}/Nc \approx 10^{-1.5}$, where $\phi_{\text{UV}} \approx 10^{18} \text{ cm}^{-2}$ is the flux of ionizing photons incident on the gas. Consequently, the dimension of the broad line region must be $\sim (10^{18} \text{ cm}) L_{46}^{1/2}$, where L_{46} is the ionizing luminosity in $10^{46} \text{ erg s}^{-1}$. For a quasar with $L_{46} \approx 1$, the broad line region has a mass only $\sim 10 M_{\odot}$ and a volume filling factor $\sim 10^{-6}$.

The broad line widths require maximum velocities $\sim 2,000$ – $10,000 \text{ km s}^{-1}$ in most objects⁴. The line ratios and equivalent widths show little dependence on line width⁵, and the line profiles of different ions in a given quasar are usually similar. The profiles of most objects are symmetrical, peaking near the systematic velocity, falling in a roughly triangular shape in the core, and curving outward in the wings but reaching the continuum at a fairly well defined velocity. A popular explanation of these profiles is that discrete emitting clouds are accelerated radially outward from rest by the radiation pressure of the ionizing continuum⁷. This results in a 'logarithmic' profile that fits a typical observed profile quite well except for the core⁸. However, to produce the observed line intensities, the clouds must be very optically thick in the Lyman and Balmer lines. As a result, hydrogen lines are emitted strongly from the ionized side of the cloud facing the continuum source, but much more weakly from the outward facing sides, which are neutral and highly opaque to the hydrogen lines. In radial outflow, this leads to asymmetries in the line profiles, with the red side of the profile being stronger than the blue side; the predicted asymmetry is extreme for $\text{Ly}\alpha$ ^{2,9}. In contrast, the observed Balmer and $\text{Ly}\alpha$ lines usually are quite symmetrical^{4,10}.

Another picture for the broad line region attempts to tie it to the popular accretion disk model for the energy source of quasars¹¹. The broad line region consists of a disk-like arrangement of clouds of gas orbiting around the central black hole, or the skin of an accretion disk photoionized by radiation from a nonthermal source^{12–14}. In either case, gas at any radius has

a circular velocity $v_{\phi} = (GM/r)^{1/2}$. To fit the line profiles, one must have a special distribution of line emission with radius, having emitting material at all speeds from $\sim 10^{4.0} \text{ km s}^{-1}$ down to $\leq 10^{2.5} \text{ km s}^{-1}$. The model has the advantage of giving symmetrical profiles because clouds on the approaching and receding sides both have their bright faces partially visible to the observer. However, the keplerian disk picture has difficulty explaining the similarity of the line profiles of different ions. The slow moving gas emitting the line peak is $\geq 10^3$ times farther from the central mass than is the gas emitting the extreme line wings. As a result, it is subjected to a much weaker flux of ionizing radiation. Unless the gas density varies as r^{-2} , the ionization parameter varies strongly with radius. Some lines will be emitted more strongly from the outer disk, and others more strongly from the inner disk. The former will have narrower profiles than the latter. On the other hand, for constant ionization parameter, $N \propto r^{-2} v^4$, and there is a huge variation in density across the line emitting region. One cannot avoid either truncating the wings of C [III] $\lambda 1,909$, or producing excessive, semi-broad forbidden lines such as [O III] $\lambda 5,007$ from the outer disk¹³. This is the so-called '[O III] problem'. None of these effects is observed.

Another way of relating the broad line gas to the energy source is to envision the clouds as falling radially into the central object. This picture has some success in producing the basic line shape¹⁵ but it falls prey both to the [O III] problem and to the prediction of strongly asymmetric hydrogen line profiles whenever systematic radial motions are involved. The asymmetry problem can be avoided by utilizing clouds on parabolic orbits that start far away from the central object, fall near it, and recede again. With equal numbers of clouds moving inward and outward, the optically thick lines are symmetric¹⁶. However, this still gives the velocity law $v \propto r^{-1/2}$, so that a large range of radii are required for a line that rises to a central peak; and the [O III] problem remains a serious objection.

These considerations pose a fundamental question: how is it possible to have circular motions that give symmetric line profiles, to have gas moving at a wide range of velocities to explain the line shape, and to have the gas contained within a small range of radius to have similar profiles for different lines?

In answer, let us consider the possibility that the broad line region consists of a layer of ionized gas on the surface of a rotating, supermassive star. The equatorial speed is set by the half-width at zero intensity, typically $5,000 \text{ km s}^{-1}$. The centrally peaked profile requires a concentration of emission towards the poles of the star's rotation. This happens naturally if we assume that the ionizing continuum is emitted in all directions from a 'jet' that extends along the rotation axis above the surface of the star. If, as we show later, the surface is rather cool, then a layer of photoionized gas will be produced whose line emission per unit area is roughly proportional to the incident flux of ionizing radiation.

I have calculated the line profile resulting from a model of such a situation. The jet was assumed to be a cone with half-angle w_j . The quantity of ionizing radiation emitted per unit length along the jet axis was allowed to vary as $dL/dz \propto (z/R)^{-p}$ where R is the stellar radius, z is the distance along the jet axis measured from the centre of the star, and $z/R \geq 1$. For $p > 1$, the jet radiates finite total power. Given the choice of p and w_j , the flux of ionizing continuum deposited per unit area

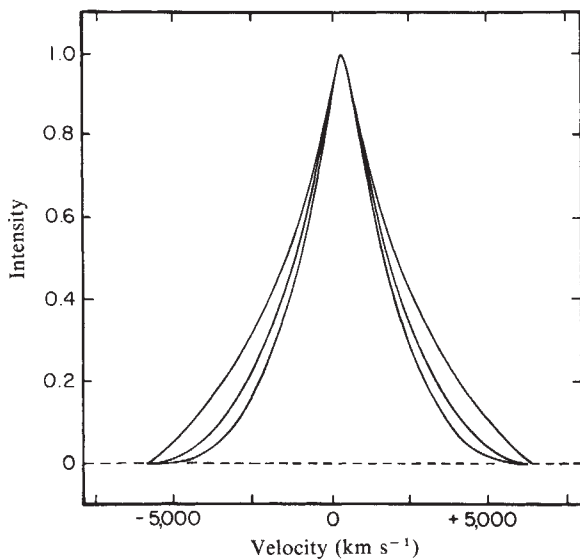


Fig. 1 Emission-line profiles for rotating spheres with $v_{\text{eq}} = 7,000 \text{ km s}^{-1}$ and $\sin i = 0.866$ excited by axial jet with $w_j = 0.1$ and $p = 0, 2, 4$ in order of decreasing width.

on the stellar surface is fixed, and the line emission per unit area was assumed to be proportional. For rigid rotation, the shape of the line profile is then determined for a given inclination of the rotation axis to the line of sight. The full width at zero intensity is $2v_{\text{eq}} \sin i$, where v_{eq} is the rotation speed at the equator; but the shape is not sensitive to v_{eq} .

For a uniform line source ($w_j = p = 0$), the rate of continuum energy deposition per unit area of stellar surface (taking into account the angle of incidence θ) is $F(\theta) = (1 - \sin \theta)/\sin \theta$, apart from a scale factor. Here θ is the polar angle of the surface point. For $p > 1$, most of the flux comes from $1 \leq z/z_1 \leq 2$, where $z_1 = (\cos \theta)^{-1}$ is the height along the jet that just appears on the horizon. Thus the above expression may be multiplied by $(z_1/R)^{-p} = (\cos \theta)^p$. Also, for a uniformly filled cone, the flux levels off for $\theta < w_j$. An economical approximate formula is therefore $F(\theta) \approx (\cos \theta)^p (1 - \sin \theta)/(\sin \theta + \sin w_j)$. This seems adequate for our exploratory purposes, especially since the profiles turn out to be fairly insensitive to p and w_j . An approximate formula was also used to estimate the finite flux of continuum at the equator for $w_j > 0$, but this had an insignificant effect on the line wings for $p > 1$ and $w_j \leq 0.2$ as we shall assume.

Figure 1 shows the computed line profile for the case of $v_{\text{eq}} = 7,000 \text{ km s}^{-1}$, $w_j = 0.1$ and $\sin i = 0.866$, the median value for a random sample. For $p = 0, 2$, or 4 , the profiles bear a remarkable resemblance to broad line profiles that are commonly observed^{4,5}. The degree of curvature in the wings increases with increasing p , and individual quasars show a similar variation. Therefore, the supermassive star picture roughly accounts for the observed profile shapes, without great sensitivity to the unknown parameter p .

Variation of the jet width $w_j \leq 0.2$ also has a minor effect on the core of the line profiles. Variation of $\sin i$ affects mainly the line width, although smaller $\sin i$ gives more curvature in the wings. Lines with different limb brightening laws¹⁷ have only slightly different profiles. Rotational broadening is consistent with the lack of a systematic dependence of line equivalent widths or intensity ratios on line width⁵. Also, the observed distribution of linewidths is consistent with rotational broadening⁵.

The line profiles of different ions will be affected by the variation of the incident radiation flux across the stellar surface. However, for $p = 2$, the flux decreases only a factor ~ 30 as one goes from $v = 0.1v_{\text{eq}}$ to $v = 0.8v_{\text{eq}}$. In contrast, in a keplerian disk, an eight times increase in velocity is accompanied by a factor of 4,000 increase in flux, if $\phi_{\text{uv}} \propto r^{-2}$. Thus, the variation

in physical conditions across the stellar surface is much milder than that which leads to the [O III] problem in keplerian geometries. For constant ionization parameter, as argued for below, the line profiles of different ions can be similar, since N (proportional to ϕ_{uv}) decreases only moderately from pole to equator. This is borne out by computations of line profiles for different ions using a grid of photoionization models supplied by G. Ferland. The decrease in N from pole to equator causes C III $\lambda 1,909$ to have a slightly larger full width at half intensity than that of C IV $\lambda 1,549$; but the difference is likely to be masked by observational uncertainties such as blending with weaker lines.

If the jet radiates isotropically, the fraction of ionizing continuum intercepted by the star is mainly a function of p . (The w_j dependence is minor for $w_j \leq 0.2$ as we assume.) For $w_j = 0$, this fraction is $\Omega/4\pi = 0.11, 0.19, 0.27$ for $p = (2, 3, 5)$; and it approaches zero for $p \rightarrow 1$ and $1/2$ for $p \rightarrow \infty$. The observed emission-line equivalent widths suggest $\Omega/4\pi > 10^{-1}$, so that $p > 2$. Because the profiles are not spectacularly dependent on p , and because the jet may emit anisotropically, it is probably not realistic to expect an observable correlation between equivalent width and line profile from object to object.

If the nonthermal continuum source is tilted with respect to the rotation axis, the line peak is shifted from $v = 0$. Rather, it occurs approximately at the radial velocity of the sub-jet point. However, the line still intersects the continuum at $\pm v_{\text{eq}} \sin i$; and this causes the profile to be steeper on the side of the peak that is away from $v = 0$. Cases of this nature are observed, with broad line peaks shifted up to $\sim 2,000 \text{ km s}^{-1}$ either blueward or redward from the narrow line velocity, but with wings more or less centred on the narrow line velocity^{4,18}. Gaskell¹⁸ has attributed these shifts to the orbital motion of a binary supermassive black hole. I suggest instead that these are cases of rotating supermassive stars with misaligned jets. For likely orientations, the peak velocity should evolve from blue to red, because the sub-jet point always first comes into view on the approaching limb. For $v_{\text{eq}} = 5,000 \text{ km s}^{-1}$, the rotation period should be $\sim 10^2\text{--}10^3 \text{ yr}$ for luminous quasars but considerably less for Seyfert galaxies, given $R_{18} \sim L_{46}^{1/2}$ as required to maintain the correct ionization parameter.

Let us now estimate some of the physical properties of the required supermassive object. For $L_{46} = 1$, we take $R \approx 10^{18} \text{ cm}$. For the keplerian velocity to exceed the equatorial speed we require

$$M \geq (10^{9.9} M_{\odot}) v_{\text{eq},9}^2 R_{18} \quad (1)$$

where $v_{\text{eq},9} = v_{\text{eq}}/10^9 \text{ cm s}^{-1}$. The gravitational redshift is

$$cz_{\text{grav}} = (300 \text{ km s}^{-1}) v_{\text{kep},9}^2 \quad (2)$$

For $v_{\text{kep}} \leq 10^4 \text{ km s}^{-1}$, the shift would be difficult to detect, particularly in view of the unexplained difference between the peak velocities of the low and high ionization broad lines¹⁹ and the narrow line contribution to the line peak. However, a limit $cz_{\text{grav}} \leq 500 \text{ km s}^{-1}$ probably can be assumed, and this implies that v_{kep} cannot exceed v_{eq} by a large factor. Hence, we conclude that, in the equatorial direction, the supermassive star is strongly supported by angular momentum, and that it has a mass of $\sim 10^{10} M_{\odot}$ for a luminous quasar.

If the remaining support is provided by radiation pressure ($aT^4 \sim GM^2 R^{-4}$), an internal temperature of $\sim 10^5 \text{ K}$ is required. This is much lower than the value required for gas pressure support, $T \sim 10^{10} \text{ K}$. Therefore, gas pressure support is negligible, since the star cannot approach the required temperature without first being disrupted by radiation pressure. However, a star upheld by radiation pressure radiates at the Eddington limit, $L_{\text{Ed}} = 4\pi GMc/\kappa$, where κ is the mean opacity per unit mass. For electron scattering opacity, this could exceed by a factor $\sim 10^2$ the assumed nonthermal luminosity of $10^{46} \text{ erg s}^{-1}$, given $M \sim 10^{10} M_{\odot}$. Continuum observations probably require $L_{\text{thermal}} \leq L_{\text{nonthermal}}$, and thus $\kappa \geq 100 \text{ cm}^2 \text{ g}^{-1}$ if the star is supported by radiation pressure. The Kramers opacity is essentially κ_{es} for the relevant conditions, and there-

fore some unknown opacity source would be required. Alternatively, magnetic support, with $B^2/8\pi \approx GM^2R^{-4}$, requires $B = 10^4$ G. (Cyclotron opacity is negligible for this field.) This may be compared with the electromagnetic luminosity produced by a rotating magnetized 'spinar',

$$L = (s/8c^3)B^2R^6\Omega^4$$

where s is an efficiency factor taken to be 10^{-1} (ref. 20). If we identify this with the 10^{46} erg s $^{-1}$ nonthermal luminosity assumed for the jet, then $B \approx 10^4$ G. The agreement with the field required for support is intriguing. However, the line profiles require the continuum to come from near the axis rather than the light cylinder.

Since v_{eq}/v_{kep} is not small, the star will be significantly flattened by rotation. In the extreme case of a rigidly rotating disk illuminated by an axial jet, the computed line profiles are quite similar to those of a rotating sphere. Presumably, profiles for an oblate spheroid will not differ greatly from those shown in Fig. 1. (Note that rigid rotation of a gaseous disk would occur in the gravitational potential of a uniform spherical mass distribution. For an isothermal sphere of ordinary stars with $M \sim 10^{10} M_{\odot}$ and $R_{core} \sim 10^{18}$ cm, the star-star collision time is very short. However, compact stellar remnants or weakly interacting particles, with masses ≥ 3 keV for fermions, might provide such a potential well. Pure rotational support would avoid the large nonthermal luminosity discussed above with regard to radiation pressure support.)

The effective temperature of the star is $T_{eff} \approx (10^{3.3} \text{ K})L_{th}^{1/4}$, where L_{th} is the thermal luminosity. (Since the surface temperature is low, there will be zones of ionization of H and He in the envelope, and this suggests the possibility of pulsational instabilities analogous to those in Cepheid variables.) This rather cool atmosphere will be photoionized on its surface and heated to $\sim 10^4$ K by the incident ionizing continuum. Above the line emitting layer, there will be a corona at $\sim 10^8$ K heated by the X-ray component of the nonthermal continuum. The boundary between the corona and the emission-line layer will occur when the ratio of gas pressure to ionizing radiation flux has a critical value given by two-phase equilibrium calculations²¹. This causes the emission-line gas to have a universal ionization parameter that is suggestively close to that required by photoionization models of the broad line region. This will occur at all points on the supermassive star, helping to assure similar line profiles for different ions. The ionizing radiation is absorbed in a layer of thickness $\sim 10^{12}$ cm, comparable with the pressure scale height at 10^4 K, so that the density remains near the critical value.

Supermassive stars were considered as a possible energy source for quasars immediately after their discovery^{20,22-24}. Since $GM/R \sim 10^{-3}$ whereas β = (gas pressure/radiation pressure) $\sim 10^{-5}$, a nonrotating star with the present dimensions would be unstable in general relativity²⁵. However, the stability problem is mitigated by rotation²⁶, and the parameters derived here are not greatly different from those considered by some workers²⁷. The idea has not become popular, perhaps partly because of a lack of any convincing observational connection. The broad emission lines may provide such a connection.

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Expected high-frequency radiation from the 1.5-ms pulsar

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The 1.5-ms pulsar PSR1937+214 has so far been observed only in the radio range¹, and X-ray and γ -ray observations at energies of between ~ 30 and 1,000 MeV have yielded only upper limits for the radiation flux²⁻³. An optical identification of the 1.5-ms pulsar has been proposed⁴, but this identification cannot yet be considered definite. Thus it is of great interest to determine in which spectral region the PSR1937+214 pulsar shows a radiation maximum. Here I consider how the current pulsar theory answers this question, and show that the 1.5-ms pulsar should be an intense source of γ quanta with an energy of $\sim 10^{11}$ eV below which γ rays freely leave its magnetosphere. The flux of superhard γ quanta emitted by this pulsar may exceed, by an order of magnitude, the flux from the pulsar in the Crab Nebula.

Each thoroughly developed theory of pulsars suggests the existence of a polar gap near the magnetic poles of a neutron star; in that gap the electric $E_{\parallel}$ component along the magnetic field is non-zero. The expected height of the polar gap is given as⁵

$$H \approx 5 \times 10^3 \left(\frac{R_c}{10^6 \text{ cm}} \right)^{2/7} \left(\frac{P}{1 \text{ s}} \right)^{3/7} \left(\frac{B_p}{10^{12} \text{ G}} \right)^{-4/7} \text{ cm} \quad (1)$$

which is about 3×10^4 cm for the 1.5-ms pulsar (where the pulsar period $P = 1.558$ ms, the magnetic field intensity at its pole $B_p \approx 5 \times 10^8$ G, the curvature radius of the magnetic field lines in the polar cap $R_c \approx (c/\Omega R)^{1/2} R \approx 3 \times 10^6$ cm, the angular velocity of pulsar rotation $\Omega = 2\pi/P \approx 4,000 \text{ s}^{-1}$, and $R \approx 10^6$ cm is the radius of a neutron star).

Particles passing through the gap are accelerated to energies of $\sim eHE_{\parallel}$ which corresponds to the Lorentz factor:

$$\gamma_b = \frac{eHE_{\parallel}}{mc^2} = \frac{e\Omega H^2 B_p}{mc^3} \approx 4 \times 10^7 \quad (2)$$

with $E_{\parallel} = (\Omega H/c)B_p$, according to ref. 5.

The maximum curvature radiation of these particles corresponds to photons with energies

$$\mathcal{E}_c = \frac{3}{2} \frac{\hbar c}{R_c} \gamma_b^3 \approx 10^{12} \text{ eV} \quad (3)$$

Photons with energy $\mathcal{E}_{\gamma}$ satisfying the condition:

$$\mathcal{E}_{\gamma} > \mathcal{E}_{\gamma}^{\text{abs}} = 10^{10} \left(\frac{B}{10^{12} \text{ G}} \right)^{-1} \left(\frac{r}{10^6 \text{ cm}} \right)^2 \left(\frac{P}{1 \text{ s}} \right) \text{ eV} \quad (4)$$

will be absorbed before leaving the pulsar magnetosphere⁶ (here r is the distance from the centre of the star to the region of photon emission). With $r = R$ for PSR1937+214 we have $\mathcal{E}_{\gamma}^{\text{abs}} \approx 3 \times 10^{10}$ eV, that is, the curvature-radiation photons with energy $\mathcal{E}_c$ will be absorbed in the magnetic field to create electron-positron pairs with energy $\sim \mathcal{E}_c/2$.

The pitch-angle of the pairs formed is $\psi \approx 10^{18.7} \text{ eV} \cdot G / \mathcal{E}_c B_p \approx 10^{-2}$ (refs 7, 8). The maximum synchrotron radiation of these particles corresponds to the photon energies:

$$\overline{\mathcal{E}}_s \approx 0.5 \hbar \omega_B \left(\frac{\overline{\mathcal{E}}_c}{2mc^2} \right)^2 \psi \approx 10^{11} \text{ eV} \quad (5)$$

where $\omega_B = eB/mc$ is the gyration frequency.

As $\mathcal{E}_s > \mathcal{E}_\gamma^{\text{abs}}$, the synchrotron radiation is also absorbed in the magnetic field, that is, an electron-positron cascade will be developed in the magnetic polar cap of the pulsar.

Since the Lorentz factor of the pairs created is $\gamma_{\pm} \approx 10^6 - 10^7 \gg 1/\psi$, the pairs will emit most of their energy. The Lorentz factor of the pairs is of the order of $1/\psi \approx 10^2$ after they have lost their energy via the synchrotron radiation.

The energy first confined to the curvature radiation will be re-distributed with the developing cascade, towards the lower energies. As a result, the radiation maximum from the millisecond pulsar magnetosphere is centred near the energy $\mathcal{E}_\gamma^{\text{abs}}$. As the developing cascade removes the γ -quanta generation region from the pulsar surface and as most of the curvature radiation is generated in the region at a distance $\sim R$ from the pulsar surface, r in equation (4) can be estimated to be $r \approx 2R$. Therefore, the maximum radiation of the millisecond pulsar should correspond to photons with energies $\sim 10^{11} \text{ eV}$.

At a distance $r \gg R$ the primary particles will have the following Lorentz factor^{9,10}:

$$\gamma = 10^7 \left(\frac{R_c}{10^7 \text{ cm}} \right)^{2/3} \quad (6)$$

which is much less than γ_b as determined from equation (2) (the quantity R_c in equation (6) is taken at the surface of the star). Hence most of the energy of the primary particle is radiated, and the γ luminosity of the pulsar may be estimated as an energy flow carried away by the primary particles through the upper surface of the polar gap:

$$\mathcal{L}_\gamma = \pi R_p^2 n_{\text{cr}} \gamma_b mc^2 \approx 7 \times 10^{33} \text{ erg s}^{-1}, \quad (7)$$

where $R_p = (\Omega R/c)^{1/2} R$ is the radius of the polar cap, $n_{\text{cr}} = \Omega B_p / 2\pi ce$.

The expected γ -luminosity is equivalent to about 1% of the total rate of the energy loss by the millisecond pulsar with the latter being $I\Omega\dot{\Omega} \approx 10^{36} \text{ erg s}^{-1}$ if the moment of inertia of the neutron star is $I = 10^{45} \text{ g cm}^2$. Most energy is carried away from the pulsar by either the intense low-frequency wave or the relativistic stellar wind with the frozen-in magnetic field.

The width of the γ radiation pattern of the pulsar is about $\Delta\varphi \approx (\Omega R/c)^{1/2} = 1/3$, that is, the probability of the Earth being within the PSR1937+214 γ radiation pattern is very high.

At the distance $\mathcal{D} = 2.5 \text{ kpc}^1$ from the millisecond pulsar, the average γ -flux with the energy $\mathcal{E}_\gamma \approx 10^{11} \text{ eV}$ may approach the value

$$\mathcal{F}_\gamma(\mathcal{E}_\gamma \approx 10^{11} \text{ eV}) \approx \mathcal{L}_\gamma / 4\pi\mathcal{D}^2(\Delta\varphi) \mathcal{E}_\gamma \approx 2 \times 10^{-10} \text{ ph cm}^{-2} \text{ s}^{-1} \quad (8)$$

This value of the flux is, approximately, an order of magnitude higher than that observed from PSR0531+21 pulsar in the Crab for the same energies^{11,12}.

Up to now we assumed in the $\mathcal{L}_\gamma$ estimates that the height of the PSR1937+214 polar gap is maximal (equation (1)). We now estimate the order of $\mathcal{L}_\gamma^{\text{min}}$ assuming, in accordance with the current pulsar theory^{5,8,13,14} that for a neutron star to be a radio pulsar, e^+ , e^- pairs should be present in its magnetosphere.

The condition of electron-positron pair production in the pulsar magnetosphere is obviously $\mathcal{E}_c > \mathcal{E}_\gamma^{\text{abs}}$ at $r = R$. With equations (3) and (4) this condition may be rewritten as: $\gamma_b > \gamma_b^{\text{min}}$ where $\gamma_b^{\text{min}} = [2\mathcal{E}_\gamma^{\text{abs}} R_c / 3\hbar c]^{1/3}$. We have $\gamma_b^{\text{min}} \approx 10^7$ for this millisecond pulsar. For such values of the Lorentz factor of primary particles the $\mathcal{L}_\gamma$ -value and, therefore, $\mathcal{F}_\gamma$ decrease by about an order of magnitude compared to the above values, thus the expected flux of hard γ quanta is of the same order of magnitude as that observed from the Crab pulsar.

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Liquid-gas phase transition in nuclear matter

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Every self-bound Fermi liquid will exhibit a liquid-gas phase equilibrium at low temperatures, because the pressure is positive at low densities due to the kinetic energy of degeneracy, and falls to zero again at the equilibrium density. Nuclear matter is seldom found under conditions of two-phase equilibrium, however: in usual nuclear reactions, a heated ('compound') nucleus is produced out of equilibrium with its surroundings, which are at a much lower temperature. The most familiar example of a two-phase equilibrium occurs in the crust of neutron stars inside the neutron-drip line¹, at temperatures of less than an MeV. In supernovas, in the crucial moments when the implosion is reversed to an explosion, densities comparable to those of neutron stars may be attained with associated temperatures of 5 to 10 MeV. An accurate knowledge of the properties of nuclear matter under these conditions is essential to the understanding of supernova dynamics². This communication shows how laboratory observations with the latest generation of nuclear accelerators can be used to infer the surface tension of the liquid-gas interface in nuclear matter—an essential ingredient of the equation of state for which a reliable theoretical model is not available.

The probability P_A of finding a cluster of A particles in a fluid is given by³

$$P_A = Z^{-1} \exp -(F_A - \mu A) / T \quad (1)$$

where $F_A = E_A - TS_A$ is the Helmholtz free energy of the cluster, μ is the chemical potential, T the temperature, and $Z = \exp PV / T$ is the partition function. Introducing the Gibbs free energies for the cluster $G_A = F_A + PV_A$ and for the gas $G_G = \mu$, we have

$$P_A = \exp -(G_A - AG_G) / T \quad (2)$$

Following Fisher⁴, we suppose that, for sufficiently large A , G_A possesses a liquid-drop expansion

$$G_A \propto G_V A + \sigma \Sigma(A) + \kappa T \ln A \quad (3)$$

where G_V is the free energy per particle in the liquid phase, σ the surface tension, $\Sigma(A)$ the mean surface area of a droplet of A particles, and κ is the critical exponent. The existence of the volume and surface terms for nuclei has long been established^{2,5,6}; the possibility of critical behaviour has recently been conjectured^{7,8}. For temperatures above the critical temperature, the leading term proportional to A gives the 'coalescence formula' observed in high-energy heavy-ion reactions⁹. At the liquid-gas equilibrium, $G_V = G_G$ and the distribution of droplet sizes is determined by the surface energy. The surface area $\Sigma(A)$ of spherical droplets is

$$\Sigma(A) = 4\pi r_0^2 A^{2/3} \quad (4)$$

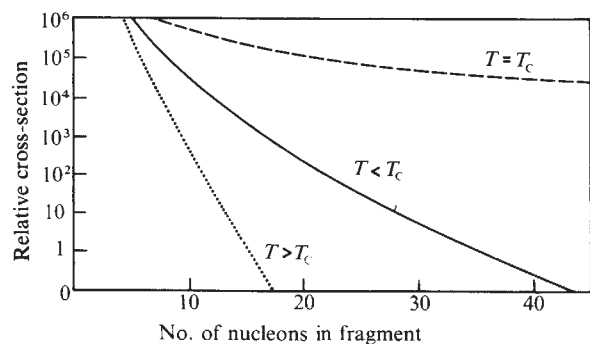


Fig. 1 Expected dependence of cross-sections on fragment mass A for temperatures above (dotted line), at (dashed line) and about half (full line) the critical temperature T_c . The critical exponent κ was 2.0, the surface coefficient was 10 MeV and the temperature was 8 MeV for the full line. The dotted line was computed with $G_V - G_G = T$ and neglecting G_S . For formulas see equations (2)–(5) in the text.

where the density of the liquid is $n = (4\pi r_0^3/3)^{-1}$. When the variation of droplet shapes is accounted for, Σ varies rather more slowly with A , typically like A^α with $\alpha = 0.6$ to 0.65 .

The coefficients σ and r_0^2 depend on the temperature. At $T=0$, r_0 is known from the density of nuclear ground states ($r_0 \approx 1.12$ fm), and σ is known from the fitted coefficient C_S of $A^{2/3}$ in the Bethe–Weizsacker mass formula ($\sigma \approx 1.14$ MeV fm $^{-2}$ for $C_S = 18$ MeV). As the temperature increases toward the critical temperature T_c , estimated^{10,11} at 15–20 MeV from nuclear matter models, r_0 increases by about one-third as the liquid density approaches the critical density n_c ; since the gas density also approaches the critical density, the density difference at the interface vanishes, and the surface tension σ approaches zero as $(T - T_c)^\gamma$, with $\gamma > 1$. Summarizing, we expect

$$P_A \approx \text{const} \cdot A^{-\kappa} \exp(-b(T)A^{2/3}) \quad (5)$$

where $b(T) \approx 18 \text{ MeV}/T$ for $T \rightarrow 0$ and $b(T) \approx \text{const} \cdot (T - T_c)^\gamma$ for $T \rightarrow T_c$; in general, $b(T) = 4\pi r_0^2(T)\sigma(T)/T$. Since microscopic or semiphenomenological models for uniform nuclear matter are well developed^{10,11}, $r_0(T)$ is not too hard to estimate; thus $b(T)$ essentially measures the surface tension $\sigma(T)$.

To measure $b(T)$ in the laboratory, it is necessary to observe the distribution P_A of clusters produced in nuclear collisions at an appropriate energy. Since the critical temperature corresponds to an excitation energy of about 25 MeV per nucleon, nuclear projectiles with laboratory energies of up to 100 MeV per nucleon are needed. To ensure conditions of uniform temperature throughout the colliding system, central collisions of equal-mass projectile–target combinations should be chosen. Such collisions lead initially to a hot, compressed fireball of nuclear matter, which expands isentropically^{12,13} until the density is so low that the supercooled system reaches the region of negative pressure and imaginary sound speed, and becomes hydrodynamically unstable¹⁴. Such an unstable, supercooled liquid may be expected to undergo a sudden, chaotic transition to a state of maximum entropy, which would, at these excitation energies, be a liquid–gas mixture equivalent to the equilibrium state.

If the target nucleus is much larger than the projectile, the explosion will occur inside the target nucleus and be hard to observe. Thus the target's mass should be similar to the projectile's. The projectile should be as heavy as possible, for three important reasons:

- (1) So that the laboratory experiment is a good model for the thermodynamic limit of large systems.
- (2) Because the liquid-drop expansion, equation (3), is only valid for large enough droplets (the Bethe–Weizsacker semiempirical formula works reasonably well for $A > 12$; for a theoretical treatment of the formation of lighter nuclei, see ref. 15);

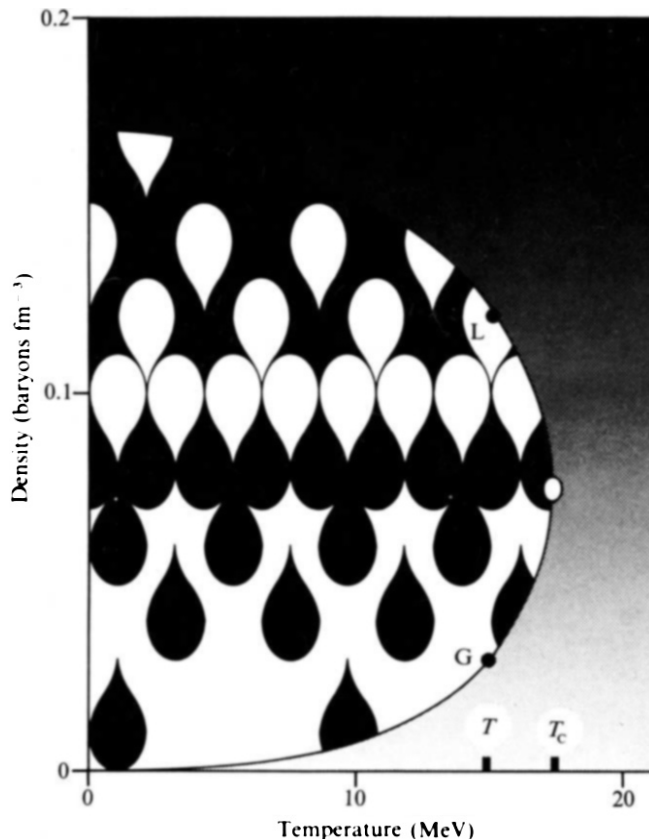


Fig. 2 Schematic phase diagram for nuclear matter. Scales of temperature and density are approximate. An open circle marks the critical point. Points G and L illustrate the gas and liquid densities, respectively, for a temperature T not far below the critical temperature T_c .

- (3) In order to obtain σ for neutron-rich matter like that in supernovae, where the proton to neutron ratio is about 0.4, compared to about 0.6 in the heaviest nuclei and 1.0 in the lighter-mass region.

The temperature may be adjusted by choosing the bombarding energy; the actual temperature attained in the final state may be inferred from observation of the spectra of protons as well as heavy fragments. The interpretation of the observed spectra will be complicated by the coulomb interactions of the reaction products, as well as by possible hydrodynamic flow of the matter at breakup^{12–14}. The expected A dependence of the cross section for various temperatures is illustrated in Fig. 1.

Once the temperature of the final state is known, the observed distribution of heavy droplets must be corrected for evaporation of nucleons, since the observed fragments will have cooled by nucleon emission before they reach the detecting apparatus. These corrections can be done using standard evaporation codes. Unfortunately, the de-excitation processes will probably make it very difficult to obtain information from droplets with $A < 12$. Even more unfortunately, they may also obscure the important information on symmetry and surface-symmetry energy which was originally contained in the isotope distributions. Fortunately, the interactions of the clusters with the surrounding nucleon gas, once they are formed, will probably not be very important, since the density of the gas phase will be very low. If the density of the gas phase n_G is less than one-fourth nuclear density, the nucleons in the gas will be essentially collisionless^{9,16}; this will be the case except very near the critical temperature, since $n_G - n_c \sim (T - T_c)^{1/2}$ (see Fig. 2). Thus it should be possible to work backwards from the observed fragment masses to the original cluster distribution P_A . For each temperature, P_A should be seen to be of the form (5), and the parameters κ and $b(T)$ extracted from the data. Ideally, κ should be seen to be independent of temperature; the limited

range of A likely to be available from observations may make it necessary to combine data of different temperatures to separate κ and $b(T)$, discussed in connection with equation (5).

Ideally, one might hope to estimate the critical temperature by seeing where $b(T)$ vanishes. This may prove very difficult, however: not only would the increasing predominance of critical fluctuations make it hard to extract $b(T)$ from the data, but the liquid-gas interactions after the phase separation has occurred may make it difficult to correct for final-state interactions.

Recently⁸, the distributions of fragment masses produced in high-energy proton-nucleus collisions have been interpreted in terms of critical fluctuations. It is interesting that those data for $A \geq 12$ can be equally well fit by equation (5) with reasonable values of κ , σ and T . The lack of an observation of T , and the implausibility of idealizing the product of a proton-nucleus collision as a uniform medium, preclude laying much weight on this observation. The heavy-ion data will be much more interesting.

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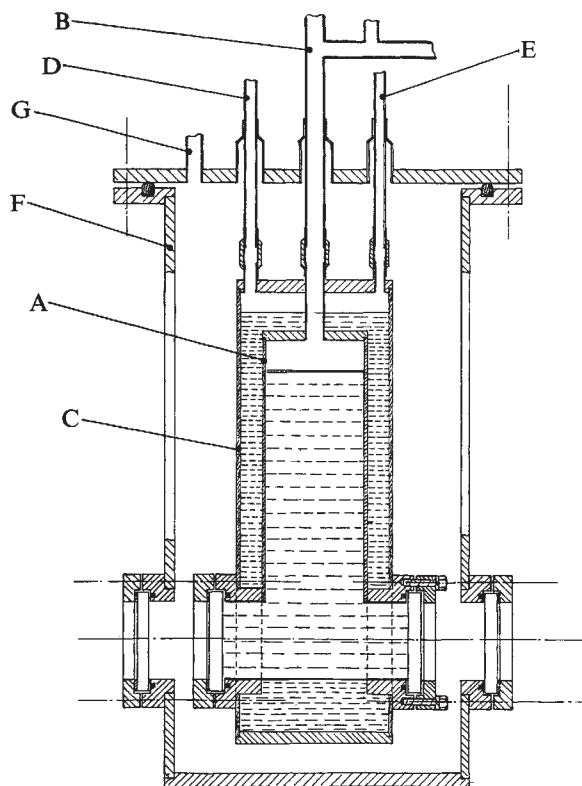


Fig. 1 Schematic diagram of low-temperature IR absorption cell: sample chamber (A) with filling assembly (B), thermal shield chamber (C) with inlet (D) and boil-off (E) tubes, and vacuum jacket (F) with pump out tube (G). The inset shows details of mounting CaF_2 windows: window (H), indium gasket (I), PTFE spacer (J), brass clamping plate (K) and brass screw with Belleville washer (L).

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The unexpectedly high solubility of water in cryogenic liquids

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The solubility of H_2O in cryogenic liquids, such as liquid nitrogen (LIN), liquid oxygen (LOX) and liquid methane, would be expected to be extremely low^{1,2}. Recent work using gravimetric methods suggested that the solubility of H_2O in liquid nitrogen could be as high as 10 p.p.m. or 10^{-5} mole fraction³. This is many orders of magnitude greater than that expected, which is 10^{-16} to 10^{-18} mole fraction (B. Jarosz, E. Szczepaniec-Cieciak and Z. Wojtaszek, personal communication). We confirm here the unexpectedly high solubility of H_2O in LIN (10.0×10^{-6} mole fraction), LOX (10.1×10^{-6} mole fraction) and liquid methane (60×10^{-6} mole fraction) at their respective normal boiling points by using Fourier transform IR spectroscopy. We have also eliminated the possibilities of experimental artefacts via parallel experiments with D_2O .

The vacuum-insulated absorption cell, used for the IR spectroscopic measurements, is illustrated schematically in Fig. 1. It is

constructed almost entirely of brass to reduce the effects of differential thermal contraction. The sample cell (A) and the enclosing thermal shield chamber (C) are suspended from the top-plate by low thermal conductivity stainless-steel tubes (B, D, E) which allow free movement when the cell contracts on cooling. The thermal shield chamber can be filled or topped-up independently so as to maintain solution samples at constant temperatures without boiling in the sample cell for long periods.

The outer vacuum jacket is sealed to the top-plate via a demountable O-ring seal and enables a pressure of 10^{-6} torr to be achieved in the vacuum space. The CaF_2 windows (38 mm diameter, 5 mm thick; H) are sealed on to the sample cell body using 1-mm diameter indium wire (I). Clamping to produce a vacuum-tight seal at low temperature is effected using brass plates (K) and brass screws with Belleville washers (L). Stress and thermal shock cracking of the CaF_2 windows is eliminated by using polytetrafluoroethylene (PTFE) spacers (J). The outer vacuum jacket windows are clamped in a similar manner except that the indium gaskets are replaced by rubber O-rings.

Before filling with liquid samples the cell was cleaned with ethanol and methylene chloride and evacuated at room temperature to test for leaks and to remove volatile contaminants. To cool the inner assembly, the thermal shield chamber was first filled with appropriate cryogenic liquid while either evacuating the sample cell (10^{-3} torr) or flushing with dry nitrogen gas. The sample cell was then filled either with an externally prepared

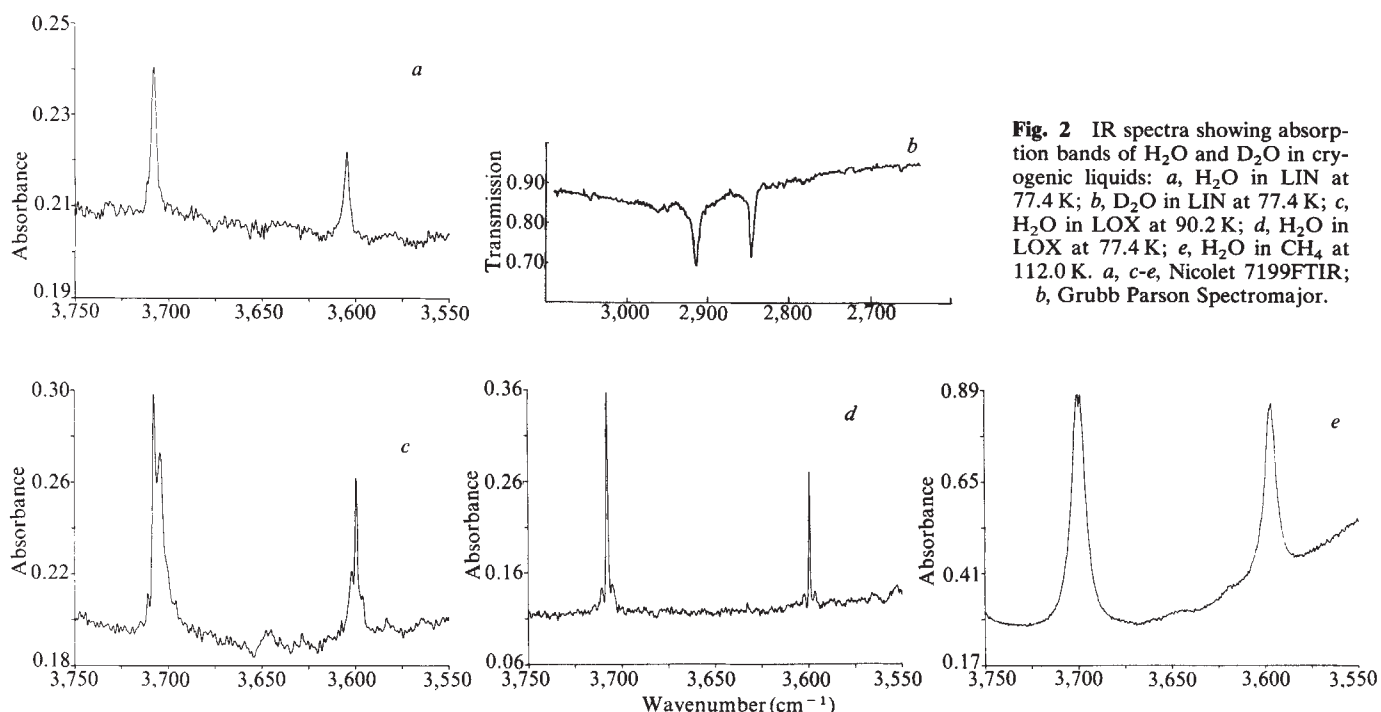


Fig. 2 IR spectra showing absorption bands of H_2O and D_2O in cryogenic liquids: *a*, H_2O in LIN at 77.4 K; *b*, D_2O in LIN at 77.4 K; *c*, H_2O in LOX at 90.2 K; *d*, H_2O in LOX at 77.4 K; *e*, H_2O in CH_4 at 112.0 K. *a*, *c*-*e*, Nicolet 7199FTIR; *b*, Grubb Parson Spectromajor.

Table 1 Measured solubilities (mole fraction) for water in cryogenic liquids

Liquid	Temperature (K)	Molar solubility
Nitrogen	77.4	10.0×10^{-6}
Oxygen	90.2	10.1×10^{-6}
Oxygen	77.4	5.8×10^{-6}
Methane	112.0	60×10^{-6}

The relative error between these figures is $\pm 4\%$. The absolute error may be as large as $\pm 50\%$.

and filtered solution or with pure cryogenic liquid and a solution prepared *in situ*.

Various methods of making solutions were tested and the simplest one for water in LIN turned out to be that in which ice particles were stirred into the liquid. The resulting solution was then filtered and transferred to the absorption cell. It was observed that the degree of saturation of the solution was determined by the rate of mixing. The resulting absorption spectra (Fig. 2*a*) showed two narrow bands (3,604 and 3,707 cm^{-1}) which correspond to the symmetric ν_1 and antisymmetric ν_3 stretching vibrations of the O—H bonds in the water molecule⁴. The spectrum of ice particles was quite distinct from these bands and occurred as a broad band in the region 3,100–3,500 cm^{-1} . This assignment was confirmed by preparing a solution of D_2O in LIN yielding spectra Fig. 2*b* with bands at 2,846 and 2,915 cm^{-1} , which correspond to the ν_1 and ν_3 O—D stretching modes of the heavy water molecule. Solutions of water in other cryogenic liquids yielded similar spectra (Fig. 2*c*–*e*) with different absorbances corresponding to different solubilities.

Calibrating the absorption cell for free molecular water at low temperatures is a major problem. Saturated water vapour has therefore been used to determine the calibration constant. Assuming this constant does not change with temperature, the measured solubilities for water are given in Table 1. Because of uncertainties in the calibration, the absolute accuracy of these solubilities may not be better than $\pm 50\%$. However, the relative error between them is much smaller and is estimated to be $\pm 4\%$.

A number of salient observations were made during these experiments. For example, LIN freshly collected from a labora-

tory liquefier and isolated from atmospheric air contained no observable H_2O but all samples of LIN, LOX and liquid methane which had been exposed to the atmosphere in open-topped containers showed considerable uptake of water. For LOX, both ν_1 and ν_3 lines were doublets whose relative intensities varied with time and temperature (Figs 2*c*, *d*). A possible explanation for these doublets is that they arise from monomer and dimer water and their behaviour suggests an equilibrium process which is a function of temperature. However, the doublet splittings ($\sim 5 \text{ cm}^{-1}$) are considerably less than those ($\sim 50 \text{ cm}^{-1}$) reported using the matrix isolation technique at 20 K (ref. 5). It follows, therefore, that estimated solubilities based on the monomer lines may be lower limits of the actual solubilities in LOX, LIN or LNG. In other work at Southampton using laser Doppler anemometry⁶, it has been observed that LIN contains large quantities of submicroscopic particulate matter. It is possible that this particulate may be ice crystals, the end-product of molecular aggregation originating from the monomer–dimer equilibrium.

The consequences of these measurements are considerable and have a bearing on all handling and storage situations in the cryogenic engineering industry and in low temperature laboratories. The uptake of water into solution in cryogenic liquids and the likely variation of solubility with pressure and temperature will result in deposition phenomena. During surface vaporization, the formation of non-volatile, molecular films of water may lead to unstable vaporization behaviour in storage conditions. The high solubility of water in liquid nitrogen may lead to desiccation of biological specimens stored for long periods of time in direct contact with liquid nitrogen.

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Effect of pressure on rare earth element partition coefficients in common magmas

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Geochemical modelling of magma generation and crystal fractionation processes at pressures corresponding to those of the lower crust¹ and upper mantle² is critically dependent on the trace element partition coefficients used in the calculations. The effects of temperature and melt composition on partition coefficients are reasonably well established^{3,4}, but the possible effect of pressure has not been closely evaluated, apart from some work on nickel partitioning⁵. Rare earth elements (REE) are important in geochemical modelling, and the need for data on the pressure effect on REE partitioning has been pointed out³. We have done a series of experiments in which the role of pressure was isolated from that of temperature and melt composition (or melt structure), and we are able to demonstrate a systematic increase in La, Sm, Ho and Lu partition coefficients for sphene/silicate liquid and clinopyroxene/silicate liquid as pressure increases from 7.5 to 30 kbar.

As part of a wider study of the role of accessory phases in magma genesis, we have taken three separate natural compositions (basaltic andesite, andesite and rhyodacite), saturated each with sphene (F-bearing), added a REE and Sr-enriched glass, and melted, then quenched, the mixes to glass starting compositions. Each of these resulting compositions have ~0.4% by weight of the oxides of La, Sm, Ho, Lu and Sr. Strontium was included as an analogue for Eu²⁺ (ref. 6). These compositions were run in a piston-cylinder high-pressure apparatus using three-capsule (Ag₇₀Pd₃₀) assemblies with 2, 5 or 10% by weight of added water and adopting routine techniques^{6,7}. Pressures ranged from 7.5 to 30 kbar and temperatures from 900 °C to 1,050 °C, with run times of 6 h at 1,050 °C increasing to 24 h at 900 °C. The run products typically consisted of sphene and clinopyroxene, ± garnet (at 16–30 kbar), ± allanite, ± chevkinite, and quenched glass. The phases were analysed for 15 major and minor elements using an ETEC wavelength dispersive microprobe⁶. To reduce errors in counting statistics for the REE, particularly in glass and clinopyroxene where the count rate is low, 10–15 analyses of each phase were obtained wherever possible. In most cases the crystals were not large enough (<8 µm) to enable separate core/rim compositions to be determined, but where larger crystals were analysed no zoning in the REE or Sr was detected. Three reversal experiments were attempted, in which natural sphene crystals were included in a REE-bearing and sphene-saturated glass starting composition. These were run for 1 and 5 weeks (hydrous) and 12 weeks (anhydrous).

Results from the synthesis experiments are shown in Fig. 1 for sphene/liquid partition coefficients ($D_{\text{REE}}^{\text{sphene/liq}}$) and in Fig. 2 for clinopyroxene/liquid partition coefficients. In both cases the partition coefficients represented for Eu²⁺ were assumed to be equal to experimentally determined values for Sr (ref. 6). The data demonstrate a significant increase in partition coefficients for each of the four trivalent REE with increasing pressure. This occurs for both the convex upward Sm, Ho (middle REE) favoured patterns for sphene, and the convex upward Sm, Ho, Lu- (middle-heavy REE) favoured patterns for clinopyroxene. Divalent Eu (Sr) partition coefficients are low and do not show a consistent variation with pressure from 7.5 to 20 kbar. Thus for sphene at 1,050 °C no significant change in D_{Sr} occurred, but at 1,000 °C a fourfold increase was observed ($D_{\text{Sr}}^{\text{sphene/liq}} = 0.05$ at 7.5 kbar and 0.20 at 20 kbar). The results

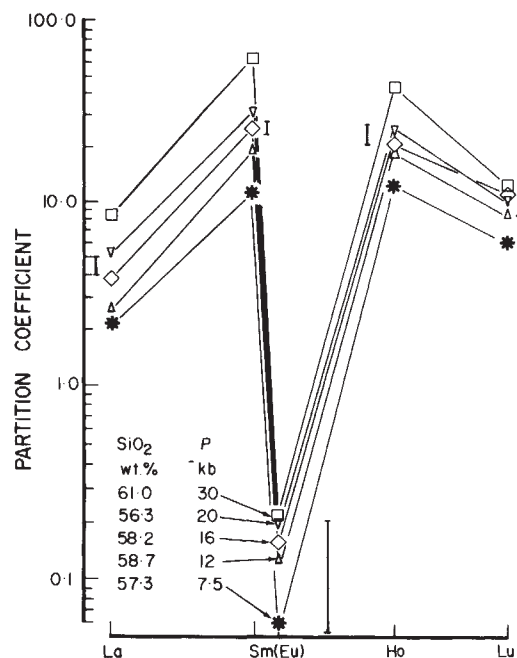


Fig. 1 Sphene/liquid partition coefficients for La, Sm, Ho, Lu and Sr (Eu²⁺) at 1,000 °C. The SiO₂ values listed refer to the SiO₂ content of the liquid coexisting with the sphene. Water at 5% by weight was added to the 7.5-, 12- and 16-kbar runs while 10% by weight was added to the 20- and 30-kbar runs. Sr partition coefficients are plotted as analogues for Eu²⁺ (ref. 6). *P* refers to the pressure of the runs in kilobars (kb). Error bars denote ±1 s.d. about the mean. The relatively large error on the Sr(Eu) *D* values is due to the very low content of this element in the sphene, and the results, although fitting the general pattern quite well, cannot be used as unequivocal evidence for the pressure effect on *D*. In this, and the following figures, the partition coefficients are plotted against REE atomic number.

for Sr partitioning between clinopyroxene and liquid were inconclusive as the Sr content of the pyroxene was generally below detection levels. Similar patterns to those in Figs 1 and 2 were observed for La, Sm, Ho and Lu in each isothermal (that is, 900, 950, 1,000, 1,050 °C) series of polybaric experiments. The results plotted in Figs 1 and 2 represent as nearly similar liquid compositions (and in turn, melt structure) as possible, using SiO₂ content as a simple measure of the melt polymerization or structure. This approximation is considered justified, rather than using more rigorous determinants of liquid structure, such as NBO/T (non-bridging oxygen per tetrahedral cation⁸), because SiO₂ is by far the dominant contributory component to the NBO/T calculation. Also for practical application in geochemical modelling, SiO₂ is the most straightforward parameter for use in governing choice of suitable partition coefficients, appropriate to the liquid composition undergoing crystallization⁹.

Strict reversals of the partition coefficients determined from synthesis runs were not obtained, due to resorption of the sphene seed crystals and/or precipitation of new sphene-containing REE on the seed crystals, rather than production of diffusion profiles of REE into the seed crystals. However, similar $D_{\text{REE}}^{\text{sphene/liq}}$ were obtained in these runs, which were carried out for times of one to two orders of magnitude greater than the routine synthesis experiments. Also the D_{REE} patterns obtained for clinopyroxene/liquid are similar to those reported by others^{3,10} and to those observed in natural phenocryst/matrix pairs (Fig. 3). Thus, it is argued that the partition coefficients obtained in this work represent equilibrium values, not affected, for example, by crystal growth rates in the relatively short-term synthesis experiments¹¹.

A second difficulty frequently highlighted in experimental work involving trace element partitioning³ centres around the

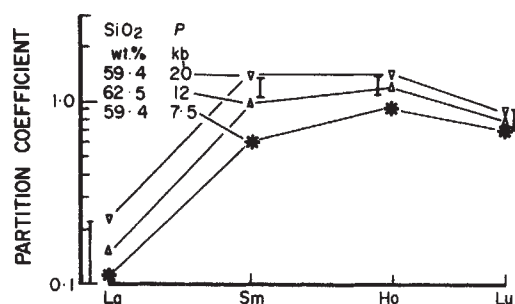


Fig. 2 Clinopyroxene/liquid partition coefficients for La, Sm, Ho and Lu. As for Fig. 1, except that clinopyroxene/liquid partition coefficients are plotted for 1,050 °C. Water at 2% by weight was added to the 7.5- and 12-kbar runs, while 5% by weight was added to the 20-kbar run. Error bars denote ± 1 s.d. about the mean. The comment for the Sr values in Fig. 1 applies to the La results in this figure.

applicability of Henry's law in experiments such as these, where a doping technique is necessary to increase the content of the trace elements to above the detection limits of the electron microprobe. This does not seem to be a problem in the present work because: (1) there is close similarity between the partition coefficient patterns observed here, and those obtained in natural systems, and in experimental systems where no artificial concentration of the REE occurred and different analytical techniques were used^{3,9,10}; (2) similar partition coefficients were obtained when half the REE content was added to the starting composition; (3) the REE content measured in some of the sphenes is close to that recorded in sphenes crystallizing from granitic magmas¹²; (4) recent work suggests that provided there is sufficient trace element concentration to saturate defect sites in a crystal, and that the trace elements substituting into 'normal' crystallographic sites dominate the observed partitioning behaviour, then Henry's law appears to hold for high concentration levels of trace elements in the system (for example up to several weight %)¹³⁻¹⁵. Thus the main result of this study—a significant and regular increase in partition coefficients for the REE with increase in pressure—is unlikely to be affected by lack of Henry's law behaviour in the experimental systems.

The actual REE content of the sphenes and clinopyroxene decreases as the degree of crystallization increases with increasing pressure, at constant temperature, so that the increase in partition coefficients for REE shown in Figs 1 and 2 cannot be attributed to any change in composition of the sphenes and clinopyroxene with increasing pressure, allowing more ready substitution of the REE at high pressure. Thus the increase in partition coefficient must be due to some change in the melt structure as pressure increases, but definition of this change is not possible from the data available. However, it is worth noting that for the REE Sm, Ho and Lu there appears to be a consistent pattern for the pressure effect and D variation with the size of the REE. Thus the increase in D with increasing pressure for $\text{Sm} > \text{Ho} > \text{Lu}$. Whatever the cause of the less favourable accommodation of REE in silicate melts with increasing pressure, it may be predicted that such causes will operate on the liquid irrespective of the crystallizing phase, and the observed increase in D_{REE} values for sphenes and clinopyroxene with increasing pressure should have general application. This is consistent with recent data suggesting similar behaviour for garnet/liquid D_{REE} , although the pressure effect could not be separated clearly from temperature and composition effects in the garnet study¹⁶.

The observed pressure effect appears to decrease slightly from andesitic liquids (~59% SiO_2) to rhyodacitic liquids (~69% SiO_2), as a threefold increase in $D_{\text{Sm, Ho}}$ for sphenes occurs in the andesite as the pressure increases from 7.5 to 20 kbar, while only a twofold increase over the same pressure range was observed in the $D_{\text{Sm, Ho}}$ for sphenes crystallizing from the rhyodacite. Inasmuch as production of analysable run products with

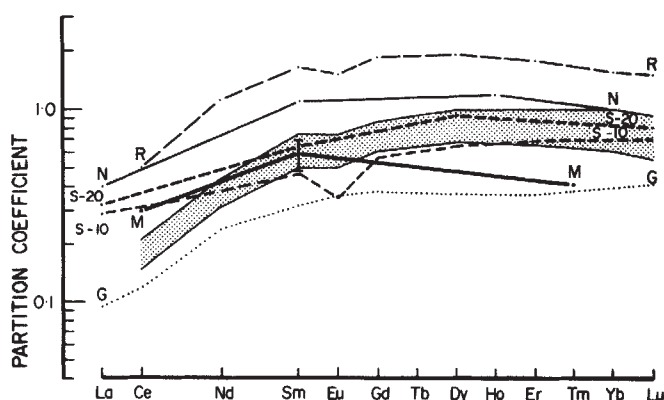


Fig. 3 Clinopyroxene/liquid partition coefficients. Natural clinopyroxenes/host magmas⁹: average to high range values (shaded area) for basaltic to andesitic rocks are plotted together, and with average rhyolitic rocks (R) indicate effect of composition and temperature. The temperature is predicted as relatively high for basaltic to andesitic rocks (for example ~1,000–1,150 °C) but relatively low for the rhyolitic rocks (for example ~900 °C), at near-surface, low-pressure conditions. Experimentally crystallized clinopyroxenes in which no artificial enrichment of REE occurred: runs at 20 kbar, 950 °C with ~49% by weight SiO_2 in the hydrous melt (M)¹⁷; 20 kbar, 1,210–1,230 °C with ~49% SiO_2 in melt (S-20)¹⁰; 10 kbar, 1,150 °C with ~50% SiO_2 in melt (S-10)¹⁸. Experimentally crystallized clinopyroxenes in which compositions were doped with REE to allow analysis via electron microprobe: runs at 15 kbar, 1,000 °C with ~56% SiO_2 in the hydrous melt (N)¹⁵; 1 atmosphere, 1,265 °C with ~60% SiO_2 in melt (G)¹⁹.

variation in pressure at near constant composition and temperature was partly achieved by varying the added water from 2 to 10 weight %, it is difficult to comment in detail on the effect of water content on the REE partitioning, except that in this water content range at least, any effect appears slight, for example D_{Sm} for sphenes is 11.2 in an experiment at 7.5 kbar, 1,000 °C, with 5 weight % added water and 57.3% SiO_2 in the liquid, while for the same pressure and temperature with 2% by weight added water and 59.3% SiO_2 in the liquid, D_{Sm} is 12.6. The slightly higher value is accounted for by the higher SiO_2 content, rather than any effect of differing water contents on the partition relations.

In conclusion, there is a real and demonstrable effect of pressure on the D_{REE} in silicate melts and this can be separated from temperature and bulk composition effects. D_{REE} increases with increasing pressure. Thus D_{REE} values determined from 1 atmosphere experiments or from natural, high-level phenocryst/matrix pairs are not generally applicable to geochemical modelling of crystal fractionation or melting processes. Different sets of D should be used, depending on whether upper crustal, lower crustal or upper mantle processes are being modelled. To some extent, the effect of pressure and of temperature on D tend to cancel out, since generally at higher pressure (giving higher D values), magmas are at higher temperature (giving lower D), because of the positive slope of silicate liquids. However, the role of pressure in REE partitioning may be of particular importance in derivation of hydrous melts produced at relatively low temperature, because of the effect of water on lowering liquids at high pressure.

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Effects of melt density on magma mixing in calc-alkaline series lavas

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We discuss here the melt density variations expected to occur during differentiation in calc-alkaline magma systems, and show how these density changes can influence the generation and chemical composition of mixed magmas. We assume that fractional crystallization of basaltic melt is the dominant process in generating the association high alumina basalt (HAB), basaltic andesite (BA), andesite, dacite and rhyolite¹. During differentiation of a basaltic parent there is an overall decrease in the density of derivative calc-alkaline liquids^{2–4}, but associated with the decline in density are density increases and related minima. At Medicine Lake Highland, petrological studies^{5–7} have identified mixed BA, mixed andesite and mixed dacite lavas. We show how liquid density variations may explain the origin of the mixed basaltic andesites and andesites at Medicine Lake. We emphasize that our mechanism may be limited to aphyric calc-alkaline suites similar to those found at Medicine Lake volcano, and we do not propose magma mixing as a general model for the origin of all andesites. Densities of residual liquids produced by differentiation of primary basaltic magmas at elevated pressures pass through a minimum, and many of the basalts and basaltic andesites in island arcs have compositions that coincide with this minimum. Fractionation at crustal pressures may create a density filter that prohibits the eruption of denser primary magmas.

The density variations produced during fractionation of a basaltic melt depend on the phases that crystallize and on their relative proportions. Figure 1 shows the individual effects of olivine, pyroxene and plagioclase fractionation on the density of an HAB. Olivine crystallization strongly depletes the melt in the high density network modifiers MgO and FeO ($\rho = 3.62$ and 5.75 g cm^{-3} respectively for oxide components in the melt⁸), and enriches the melt in the low-density network formers SiO₂, Al₂O₃ and Na₂O ($\rho = 2.24$, 2.70 and 2.22 respectively). As a result liquid density decreases dramatically when olivine crystallizes. Pyroxene fractionation removes less MgO and FeO from the melt than olivine fractionation causing a gentle decline in the densities of residual liquids. Plagioclase crystallization enriches the melt in FeO and MgO while depleting it in Al₂O₃ and CaO, producing a strong density increase. Thus the proportions of olivine, pyroxene and plagioclase in the crystallizing assemblage determine whether the densities of residual liquids increase, decrease or remain constant. As an example, the crystallization paths and density variations of two calc-alkaline lavas will be discussed in terms of plagioclase-saturated low-pressure phase relations derived from 1-atm experiments⁸ (Fig. 2). Liquids from crystallization experiments performed on an HAB and a BA were used to construct the paths shown in Fig. 2, and their compositions are reported in Table 2 of ref. 8 (see runs on 79.35g [HAB] and 79.38b [BA]). Densities of the

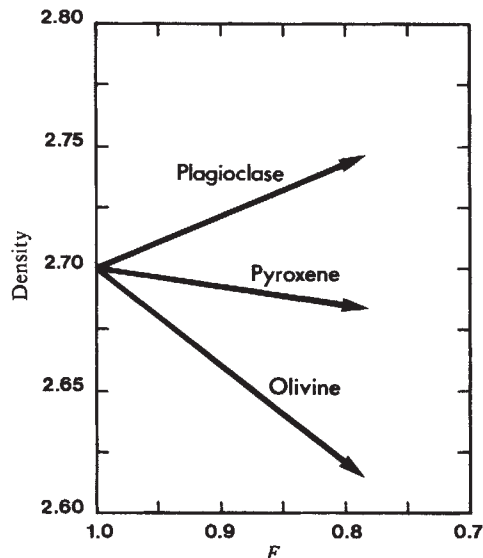


Fig. 1 Densities of residual liquids as a function of olivine, pyroxene and plagioclase fractionation. A fractionation calculation removed a single phase in 5% increments from an HAB (79-35d, Table 2; ref. 7). Densities of the residual liquids were calculated by the method of Bottinga and Weill^{3,9}. Distribution coefficients for olivine and pyroxene (Mg/Fe) and plagioclase (Ca/Na) are from ref. 8. F is the remaining fraction of liquid. Augite, pigeonite and orthopyroxene produce nearly identical changes in the densities of residual liquids. Also, a wide range in the Ca/Na distribution between plagioclase and liquid produces very similar density variations.

experimentally produced liquids (Fig. 3a) were calculated using the method of Bottinga and Weill^{3,9}.

Crystallization of the HAB at 1 atm begins with olivine + plagioclase as liquidus phases. When augite joins the crystallization assemblage, the residual liquid moves down the cotectic, and intercepts reaction point A where olivine + liquid react to form augite, plagioclase and pigeonite. The calculated liquid densities decrease as olivine (25%) and plagioclase (75%) crystallize (Fig. 3a). Although olivine is the subordinate phase, the net effect is a density decrease in the residual liquid. When augite joins the assemblage, the density levels out. Plagioclase and augite now dominate the density changes produced during crystallization, and the density remains constant or increases slightly. When liquids reach reaction point A liquid density increases dramatically. At the reaction point olivine dissolves adding dense network modifiers (MgO and FeO) and less dense SiO₂ to the melt in 2 to 1 proportions, while low-Ca pyroxene crystallizes removing these components in 1 to 1 proportions. The net result is to produce a dense iron-enriched liquid at a constant SiO₂ value. (The term 'reaction point' is used to describe the piercing points in the projected system, which are multicomponent reaction relations that should be viewed as the projection of reaction curves, since FeO, MgO and minor element contents of residual liquids are changing as the reaction progresses.)

The second liquid line of descent (Fig. 2, Modoc BA) has olivine and plagioclase as liquidus phases. As crystallization continues the liquid intercepts the olivine–orthopyroxene reaction curve and reaches reaction point B, where olivine + liquid react to orthopyroxene, pigeonite and plagioclase. In this composition magnetite appears at point B, olivine dissolves, and continued crystallization follows the orthopyroxene–pigeonite reaction curve to silica-enriched compositions. During olivine + plagioclase crystallization the density of the melt decreases (Fig. 3a). When the reaction curve and reaction point B are reached, density increases because the net effect of olivine dissolution is to add more MgO and FeO to the melt than are removed by pyroxene crystallization. The cessation of olivine reaction and the crystallization of magnetite cause a decrease in density.

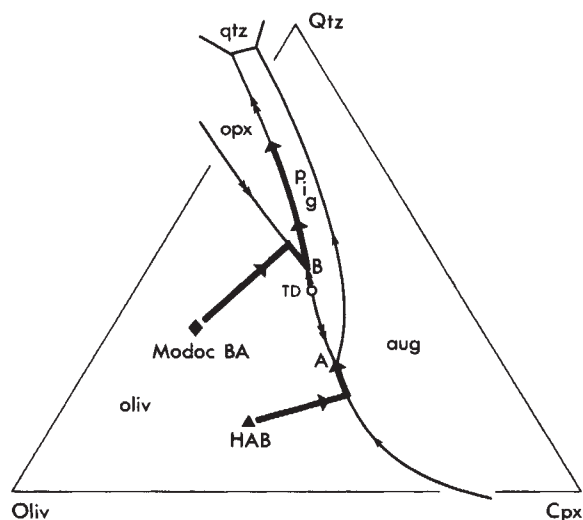


Fig. 2 Phase relations for Medicine Lake compositions at 1 atm. The primary phase volumes, cotectics and reaction curves are plagioclase saturated, and are projected from plagioclase onto the plane olivine (Oliv)–clinopyroxene (Cpx)–silica (Qtz). The projection scheme of Grove *et al.*⁸ has been modified to oxygen-normalized components, which are more representative of relative volumes of liquids. Oxygen units are calculated by multiplying the molar mineral components by the number of oxygens in their formula and renormalizing. Liquid lines of descent for two lavas are also shown. The HAB approaches reaction point A on crystallization, while the BA approaches point B. Density minima are encountered during crystallization of each liquid. See ref. 8 for liquid compositions used in constructing this and Fig. 3.

The effect of H₂O that may have been present in the magma may be estimated by extrapolating molar volume data from the system albite–water¹⁰. Because H₂O acts as an incompatible element during differentiation, its effect would be to lower melt density as differentiation proceeded. Assuming a molar volume of 26 cm³ mol⁻¹ for H₂O (ref. 10), the density minimum and its associated upturn would persist at reaction point A for initial water contents in the basalt parent of up to 1.8 wt%, which would be increased to 3.6 wt% by the crystallization required to move the liquid to reaction point A. At reaction point B the density minimum and upturn will be retained for basalt parent melts containing up to 0.5 wt% H₂O. Higher water contents would result in a steady decrease in density during differentiation.

The densities of mixed basaltic andesites and andesites from Medicine Lake Highland are plotted in Fig. 3b and were calculated using whole-rock analyses^{11–15} of sparsely phryic lavas assuming all Fe as FeO. When compared with the experimental liquids (Fig. 3a), the mixed lavas are lower in density, but plot in the vicinity of the two density minima located by the experiments (Fig. 3b). In Fig. 4, the compositions of the Medicine Lake lavas are projected onto the 1-atm phase diagram. The mixed lavas (BA and PA) are displaced to the left of reaction points A and B and fall in the olivine+plagioclase phase volume. We suggest that the compositions of the mixed lavas are locating the positions of reaction points A and B at elevated pressures. In general, increased water pressure destabilizes plagioclase while increased total pressure shrinks the olivine primary phase volume and enlarges the orthopyroxene phase volume¹⁶. These changes could move point A and B towards the projected positions of the Medicine Lake mixed lavas. We propose that the density–composition variations experienced by liquids as they approach reaction points A and B at elevated pressure would be similar to the 1-atm density changes in spite of the decrease in proportion of plagioclase to olivine+pyroxene in the crystallizing assemblage. In other words, an important control on the density relations occurs in the vicinity of the reaction points, where the dissolving olivine adds more dense network modifiers (MgO and FeO) to the residual liquid,

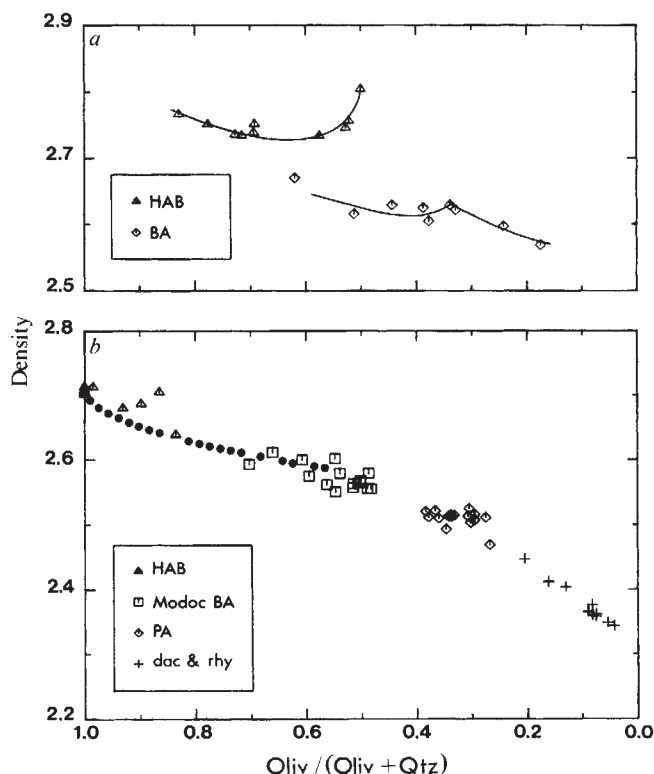


Fig. 3 Liquid densities of experimental liquids and Medicine Lake Highland lavas. Liquid densities were calculated using the method of Bottinga and Weill^{3,9}, assuming all iron as FeO and the appropriate temperatures. Liquid density is plotted against the normalized Oliv/(Oliv + Qtz) coordinate from Figs 2 and 4. The value of Oliv/(Oliv + Qtz) decreases with advancing fractionation. *a*, Density variation during crystallization displayed by experimental liquids from Fig. 2. *b*, Calculated densities of Medicine Lake mixed basaltic andesites and mixed glassy andesites are plotted along with HAB, dacite and rhyolite lavas. The dotted line is the density variation expected from the fractional crystallization model of Grove *et al.*⁸.

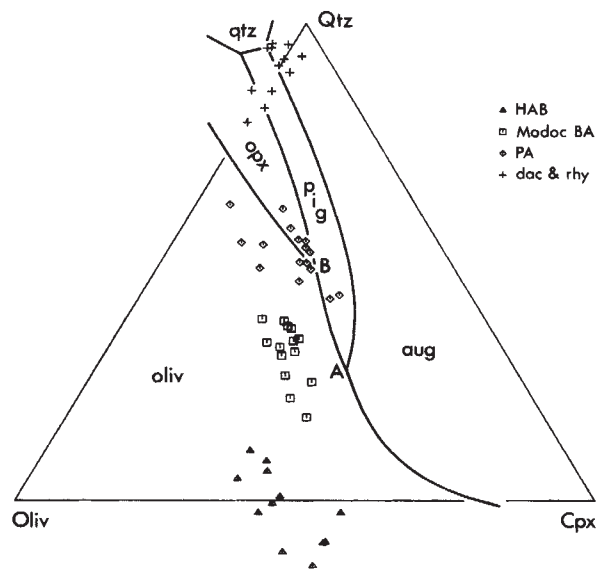


Fig. 4 Compositions of Medicine Lake Highland lavas projected into the pseudo-ternary olivine–clinopyroxene–silica. HAB is taken as representative of primary magma at the volcano. The mixed BA and mixed andesite are plotted along with more differentiated rhyolites and mixed dacites.

than are removed by the crystallizing Mg-rich pyroxene. The result is an increase in the density of the liquid.

The compositions of mixed BAs at Medicine Lake lie near the density minimum encountered as liquids approach point A (Fig. 3). We suggest that these lavas formed by fractionation of olivine-augite-plagioclase from an HAB parent. Fractionation of the three-phase assemblage in the proportions required to satisfy major element variations causes a decrease in density of derivative liquids (see model calculation, Fig. 3b). The model proportions are not the same as those derived from the 1-atm phase diagram and reflect crystallization at elevated pressure. Increasing pressure reduces the proportion of plagioclase to olivine + pyroxene in the crystallizing assemblage allowing the light feldspar component to remain in the melt. This explains the decrease in the calculated densities of the model and the lower densities of the lavas compared with the 1-atm experimental liquids. When the high-pressure equivalent of point A is approached, further crystallization causes an increase in density. If a liquid that advances to and progresses beyond the minimum that precedes the reaction point is contained in a magma chamber, and if this chamber is replenished at its base by hotter, denser parental magma, the parental magma will be trapped below the lighter cap of fractionated melt. When the underlying magma has cooled, crystallized and become equal in density to the overlying liquid, the two can mix to produce the mixed Modoc BAs. Thus, the density minimum enables two different compositions, one less evolved than the other to have the same density and to mix. One would expect the phenocryst assemblages found in the mixed magma to reflect the similarity of the two lavas. However, the phenocryst assemblages and trace element characteristics^{7,8} suggest that a very silicic component is one endmember of the mixture. Thus, mixing associated with the density minimum may destabilize the magma reservoir and trigger catastrophic mixing with lighter silicic melts that are present at the top of the reservoir.

The second composition of mixed magma is andesitic and associated with point B. We propose that these lavas formed through a similar process of fractionation described above, but the fractionation-depth conditions differed in such a way that liquids approached the high-pressure equivalent of point B. As a result of the curvature of the liquidus phase volumes in the vicinity of point B, mixing at the density minimum encountered as liquids approach point B produces superheated liquids⁷. The glassy textures and scarcity of preserved phenocrysts in Medicine Lake andesites are consistent with some of these magmas having been superheated mixed melts.

Thus, phase equilibria suggest that there are two possible compositional regimes where magma mixing could occur in calc-alkaline systems. One of these compositions is in the range of basaltic andesite, and the other is in the range of andesite. The density minimum that corresponds to basaltic andesites is analogous to the ocean floor basalt density minimum^{17,18}, differing in that it has been shifted by conditions of P , T and P_{H_2O} that exist in the upper crust. Many of the most mafic lavas identified in island arc settings have compositions similar to those that define this density minimum at Medicine Lake (52 to 54 wt% SiO_2 , 6% MgO , 18% Al_2O_3) and some authors¹⁹ have suggested that these are primary magmas. Some of these lavas could be the density filtered derivative lavas that exist as the most magnesian rock in the island arc, because they form a density cap in shallow level magma chambers through which denser primary magma from the underlying mantle source cannot rise.

Like recent discussions of magma mixing^{4,20}, ours shares the disadvantage that it is difficult to assess quantitatively. We have suggested that the existence of density minima are important, but we have not shown that density changes are capable of triggering mixing and homogenization²¹. We recognize the need for such a critical evaluation in silicate systems.

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Seismological evidence for shallow crystalline basement in the Southern Uplands of Scotland

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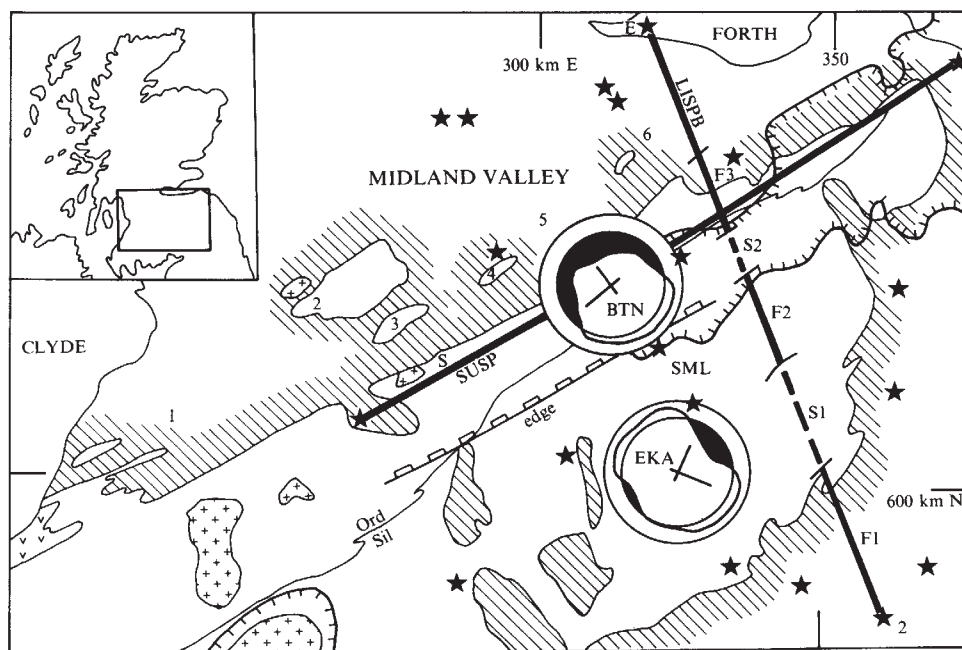
New evidence is presented, and old data re-appraised, to indicate that the Southern Uplands of Scotland contain crystalline rocks of continental affinity at shallow depth (1–5 km) in at least two zones roughly parallel to the Caledonoid strike. This is in contrast with earlier interpretations (especially of the LISPB seismic profile^{1,2}) which suggested that the lack of such rocks implied a major discontinuity across the Caledonides near the Southern Uplands Fault. The new interpretations suggest that basement indistinguishable seismologically from that below the Midland Valley continues 15–20 km southwards below the Southern Uplands, and that another such crustal block underlies the Eskdalemuir (EKA) seismological array further to the south. The existence of such blocks radically affects plate tectonic reconstructions of the Caledonides especially the margins of the Iapetus Ocean^{3–6}. Only drilling is likely to show whether the contact between basement and the overlying Lower Palaeozoic greywackes and shales is structural, igneous or, least likely, simple overstep.

The argument falls into two parts: first, presentation of the evidence of rocks of high seismic velocity ($V_P \geq 6 \text{ km s}^{-1}$) at shallow depth and second, discussion of the nature of such rocks. The new evidence is summarized, together with other relevant information, in Fig. 1.

The Southern Uplands Seismic Profile (SUSP)⁷ shows a modest lateral variation in the seismic velocity layering, but typically indicates a P-wave velocity of 6 km s^{-1} at about 1 km depth increasing to 6.3 km s^{-1} at 3–4 km depth. Observations of quarry blasts and other local seismic sources at the Broughton array (BTN), which is centred in the middle of the SUSP, show similarly high apparent velocities from sources along strike (parallel to SUSP) and in the Midland Valley to the north and west, but low apparent velocities ($5.6\text{--}5.8 \text{ km s}^{-1}$) from sources at similar ranges to the south and east. This pattern does not have the symmetry required of a simple anisotropic solution, but suggests that the high-velocity crust of the Midland Valley continues south of the Southern Uplands Fault but deepens

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Fig. 1 Map of the Southern Uplands of Scotland. Area of Lower Palaeozoic rocks is limited by shading, pluses indicate late granites, V indicates Ballantrae igneous complex. Lower Palaeozoic inliers within the Midland Valley are Straiton (1), Lesmahagow (2), Hagshaw (3), Carmichael (4), Tinto (5), North Esk (6). Inliers 2–4 are used to define basin edge (line with castellations). A line is drawn to separate Ordovician (Ord) and Silurian (Sil) outcrops. The area within which Bouguer gravity is negative is bounded by line with edging on negative side. On the LISPB profile, E and 2 identify shot positions and F1–3, S1–2, locate high- and low-velocity blocks described in the text. SUSP is the Southern Uplands Seismic Profile. Seismological arrays at Eskdalemuir (EKA) and Broughton (BTN) were used to observe seismic events at the black stars. Polar plots are of apparent velocity versus direction to source with scale rings at 5.5 km s^{-1} (outer) and 6.0 km s^{-1} (inner), so that black area defines sector in which velocity is greater than 6 km s^{-1} . The Spango granodiorite is marked S, and St Mary's Loch is at SML. Inset shows location of area.



rapidly to the south-east of SUSP and BTN. A strong gravity gradient down to the south-east supports this contention⁸.

Early work by Jacob⁹ has been followed by observations of local sources at the Eskdalemuir array (EKA) showing high-velocities along strike and low velocities at corresponding ranges across strike both to NW and SE. Such symmetry might be compatible with small-scale anisotropy, but anisotropy of about 15% ($\Delta V_{\text{max}}/V_{\text{mean}}$) requires either an extremely strong, and regionally-consistently-oriented, mineral fabric in a metamorphic rock, or a sandwich of thin low and high velocity layers, the velocity of the latter having to be unrealistically high ($V_p \approx 8 \text{ km s}^{-1}$) for such shallow depth. A solution more simply compatible with the above evidence is that a high-velocity block, 10–20 km wide, underlies EKA and extends NE–SW (along strike). The low-velocity block to the NW would be the same as that inferred to lie SE of BTN and SUP.

Re-examination of the LISPB data² (Fig. 2) shows a series of low (5.6 km s^{-1}) and high (6 km s^{-1}) velocity segments observed in the time–distance plots of shots immediately to north and south. In the absence of other data, the obvious interpretation is that the changes of apparent velocity reflect structure on a shallow refractor of velocity 5.8 km s^{-1} —the LISPB model. We show in Fig. 2 how shallow high-velocity blocks (F) with intervening low-velocity material (S) may produce similar observations. Our modelling here is at a preliminary stage: more data are required as input to a refined ray-trace modelling. We already suspect that the steep south face of F3 must dip to the NW. There are some mismatches; F2 on LISPB (Fig. 1) does not lie exactly along strike from EKA as might have been expected and curved boundaries of F2 along strike are required for it to underlie EKA. A refinement which overcomes this mismatch is to place a further high velocity block within S1—this would also give an improved fit to the LISPB data (D. K. Smythe, personal communication). The gravity field fits well qualitatively with F3 and S2 but not so well with F2 and S1; perhaps the sides of F2 dip to the south-east.

While further work is needed to locate their along-strike persistence and to determine the form of their sides we claim to have established clearly that there are major blocks within the Southern Uplands containing substantial quantities of rock at shallow depth characterized by $V_p \geq 6 \text{ km s}^{-1}$. Figure 3 provides some lithological implications of these data. Field and laboratory measurements on Lower Palaeozoic greywackes¹¹

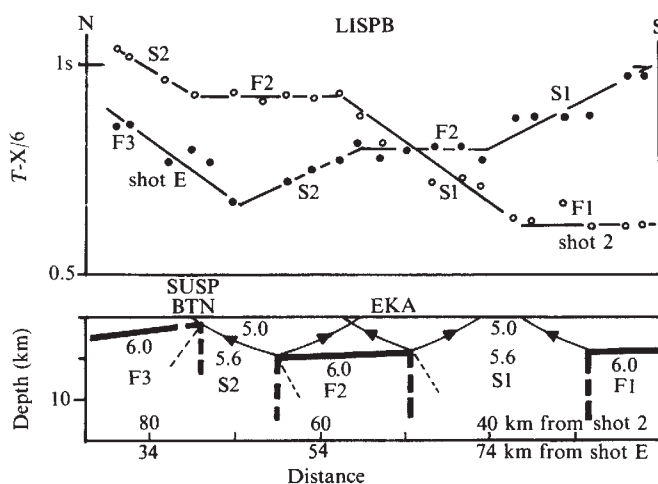


Fig. 2 Time–distance plot of LISPB data across the Southern Uplands from shots E and 2, using a reduction velocity of 6 km s^{-1} in plotting time. The structural model shows a velocity distribution containing fast (F) and slow (S) blocks which give rise to the corresponding segments of the plots, with offsets shown by arrowed ray-paths.

agree with across-strike velocities (observing S1 and S2), but are significantly lower than high velocities in F2 and F3. More mafic greywackes than those measured do exist within the Southern Uplands but are not expected to have velocities more than 0.2 km s^{-1} higher than those measured. The only locally-exposed crystalline rocks are plutonic: the velocity of a sample of the Spango granodiorite is plotted in Fig. 3 together with estimates of the velocity of equivalent rocks of more acid and more basic compositions. From this it may be concluded that F3 could be an igneous or metamorphic rock of broadly granodioritic to dioritic composition. More basic rocks are allowed provided they are more highly cracked and/or altered than the Spango sample. An alternative model to explain the high velocities along strike is that of thick sandwiches, in the two zones, of basic rocks among greywackes—perhaps slices of ocean crust under the greywackes caught in accretionary wedges. Models involving basic rocks suffer from the lack of evidence of predictably strong magnetic signatures.

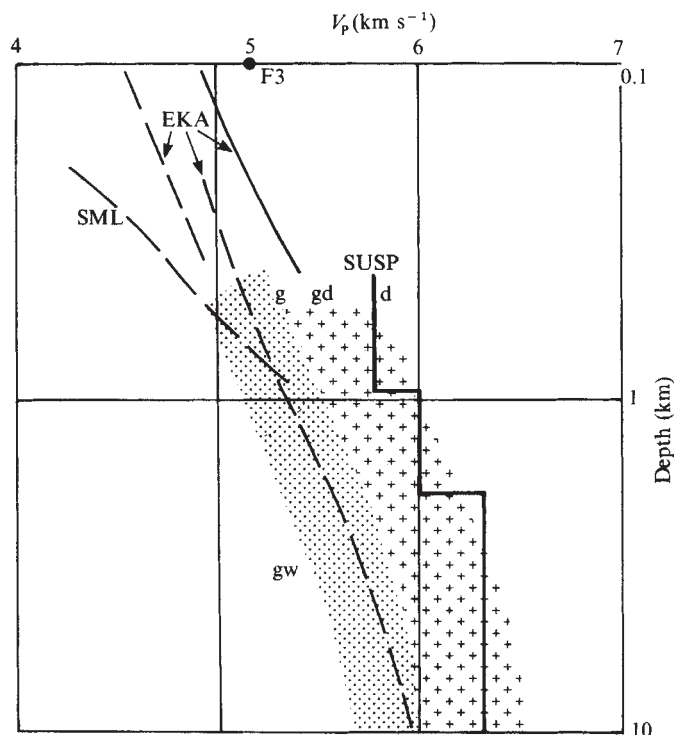


Fig. 3 Velocity-depth distributions for the Southern Uplands (note: depth scale is logarithmic). Solid lines show along-strike velocities and dotted lines cross-strike velocities. EKA curves combine observations at short range (2 shallow-depth curves) and longer range dominantly across strike. SML is from an array study at St Mary's Loch in slow belt S2. F3 is from a shallow refraction survey in the northern fast belt. Dotted area (gw) shows range of velocities measured in Lower Palaeozoic greywackes. Plusses indicate range of velocities in plutonic rocks from measurement on Spango granodiorite (gd) with estimates for diorite (d) and granite (g). SUSP, Southern Uplands Seismic Profile.

Although the existence of continental basement below the Southern Uplands has been disputed¹²⁻¹⁶, there are two geological lines of evidence which tend to corroborate our interpretation. First, many xenoliths found in vents bordering the region have continental crustal affinities¹⁷ and second, three conglomerates in the Silurian inliers of the Midland Valley (Fig. 1)¹⁸ were deposited as alluvial fans with a source to the SE, that is, in the region which is now the greywackes of the Southern Uplands accretionary prism. The lowest two conglomerates contain mainly plutonic, lava and quartzite clasts and are therefore not likely to have their source in the present Southern Uplands but rather the basement which underlies them. Using rates of clast size decline the basin margin for these two conglomerates is independently estimated¹⁹ to be roughly at the point of the basement high (F2, Fig. 1). The upper conglomerate in comprising mainly greywacke could have had its source in an uplifted accretionary prism and may therefore record the emplacement of the Southern Uplands as it was translated NW over its basement.

This interpretation differs from that of Leggett *et al.*²⁰ who prefer oppositely-directed NW translation of basement from the SE below the Southern Uplands. There is strong evidence from the 'WINCH' deep seismic reflection profile²¹ that such movement took place on a very deep NW-dipping thrust overlain by a thick crust merely capped by the remnants of the accretionary wedge. That thick crust contains our model so there is no geometrical problem, and neither is there any necessity to invoke major strike-slip movements to explain these data. A drill hole suitably sited would provide a cheap test of our thesis.

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Geochemical evidence of a massive slide in the southern Norwegian Sea

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During the 1981 cruise of the MS Tyro to the southern Norwegian Basin several piston-cores were collected for sedimentological research. Some of these were available for geochemical study (Fig. 1). In laboratory analyses the pore water composition of one of these cores showed evidence of input of non-saline water, whereas pore water in other cores from the same Basin did not give any indication of dilution with fresh water. Careful examination of the chemical constituents of the pore water and some parameters of the sediment, gives convincing evidence for the origin of the fresh water. The presence of non-saline water can be attributed to a massive slide of a coastal fresh-to-brackish sediment 'slab', with preservation of structural and pore water composition. This sliding is thought to have taken place about 12,000 to 15,000 years BP.

To obtain reliable results on pore water composition requires much care to be taken in handling the samples and in controlling temperature and oxygen conditions during squeezing¹⁻¹¹. Therefore we developed the following shipboard routine. Immediately after core collection the PVC inner cores were cut into 100 to 150 cm pieces; the pieces were then tightly sealed with plastic tops and tape and stored at 3-4 °C. Pore water extraction was started within 24 h of core collection. After lengthwise splitting of the PVC inner core into two parts, one part was immediately transferred to a nitrogen filled glove bag. The glove bag was flushed three or four times with nitrogen gas and the bag end closed to allow a slight overpressure and flux of nitrogen. The pH of the sediment was measured and the upper few millimetres were removed before samples were taken. These sediment samples were put into a glove box connected to the glove bag via a sluice system. Oxygen conditions in the glove box were monitored and were always below 0.04%.

Pore water was extracted at *in situ* temperatures (0-1 °C) in modified Reeburgh presses¹², using the same quality of nitrogen gas as used for flushing of the glove bag and box ('high purity', less than 0.001%). The squeezers were made of Delrin, with a Teflon supporting screen. Six squeezers were operated simultaneously, with pressures up to 15 bar. Cellulose acetate 0.2 µm

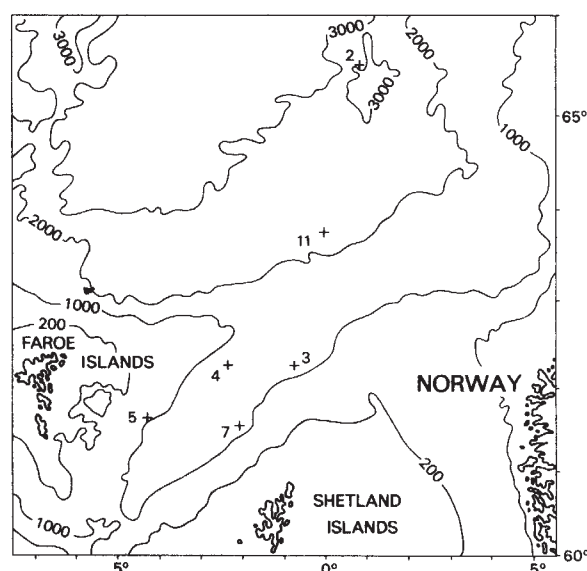


Fig. 1 Piston cores available for pore water extraction (after van Weering, unpublished).

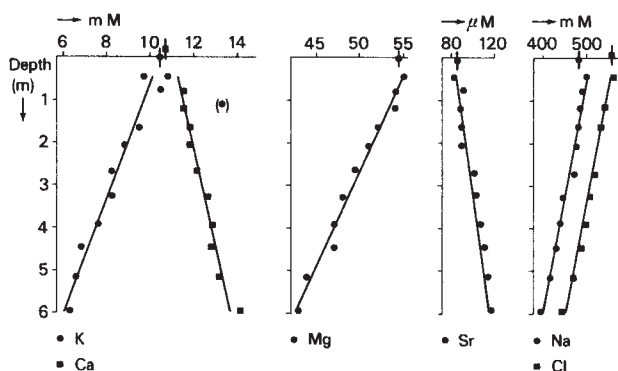


Fig. 2 K, Ca, Mg, Sr, Na and Cl concentration versus depth in core NA81-11.

membrane filters were used. After pore water extraction, the water samples were immediately subdivided into three portions to be deep-frozen, acidified and for shipboard analyses.

In one of the sampled piston cores (NA81-11) large gradients of chloride as well as alkali metals and strontium were found (Fig. 2). Except in highly anoxic cores^{13,14,20} or Deep Sea Drilling Project cores^{15-19,21-23} practically no concentration differences with depth can be observed for the alkali metals in deep sea cores. Similar gradients of K, Ca, Mg, Na, Sr do occur in some of the reported cores, but it has been possible to explain them by diagenesis, formation or structural ordering of minerals, hydrothermal input, tectonic movements, occurrence of gashydrates, or input of meteoric water^{6,10,24,25-28}. In the case of core NA81-11, however, there are at present only intermediate anoxic conditions, as is shown by the low nitrate ($<1 \mu\text{mol l}^{-1}$), as well as ammonia concentration ($<600 \mu\text{mol l}^{-1}$) and alkalinity ($<5 \mu\text{eq. l}^{-1}$). Only rarely have chloride changes with depth been found in oceanic sediments^{17-26,29-34}. The approximately linear decrease with depth of Cl and Na, Mg, K (Fig. 2) and a constant ratio of these elements and chloride with depth (Fig. 3) indicate a dilution effect. Ca and Sr concentrations increase with depth as does their ratio to chloride (Fig. 3). The Ca/Sr ratio, however, virtually remains constant. A similar phenomenon was observed by Suess^{35,36} in the Baltic at a waterdepth of 20 m and by Manheim *et al.*^{24,26} in the Black Sea. Both explained the behaviour of Na, K, Mg and Cl by dilution and the behaviour of calcium and strontium by exchange reactions with clay minerals: clay minerals deposited in a fresh-water environment are predominantly in the Ca-form, whereas

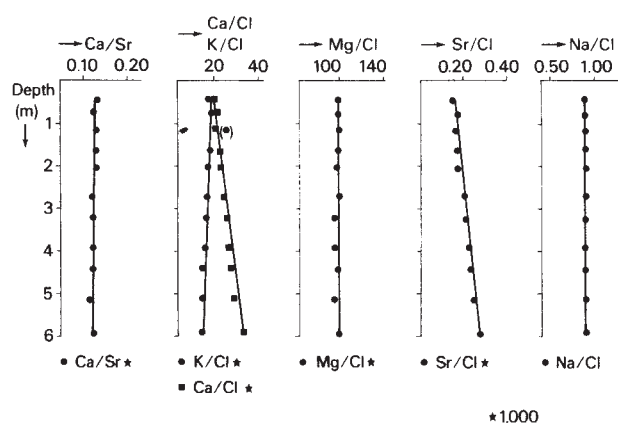


Fig. 3 Ratios of Ca/Sr, K/Cl, Ca/Cl, Mg/Cl, Sr/Cl and Na/Cl versus depth in core NA81-11. All ratios, except Na/Cl have been multiplied by 1,000.

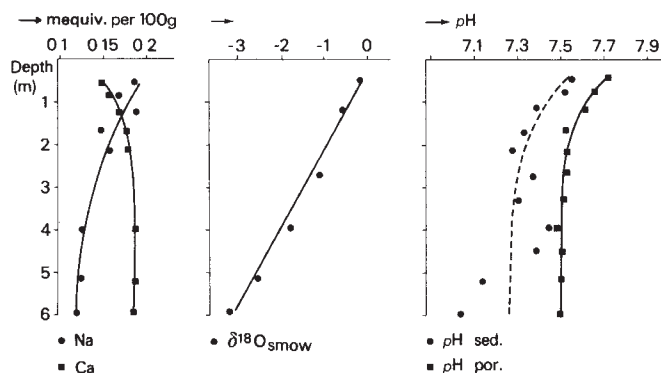


Fig. 4 a, Exchangeable Na and Ca in core NA81-11; b, $\delta^{18}\text{O}$ relative to SMOW (standard mean ocean water): $((^{18}\text{O}/^{16}\text{O})_{\text{sample}} / (^{18}\text{O}/^{16}\text{O})_{\text{smow}} - 1) \times 1,000\%$; c, pH (punch in) values for core NA81-11.

those in the marine environment are mostly in the Na, Mg, K form. The pore water composition, the pH (Fig. 4c), and the exchangeable alkali metals of this core all indicate (Fig. 4a) that the sediments in the lower part of the core were deposited in a fresh-to-brackish water environment. The oxygen isotope composition of the pore water (Fig. 4b) points in the same direction. If the relative percentage of fresh water is determined on the basis of chloride concentrations in the pore water, one can estimate the oxygen isotope composition of the fresh water. The chloride profile in this core seems to be controlled by diffusion, and since the diffusion-coefficient for H_2^{18}O is much higher than for chloride¹⁷, a more realistic value will be even more negative. The value found ($-16\% \pm 1$) is rather low for these latitudes^{37,38}, so influx of glacial water with a relative depletion in ^{18}O is probable. Data on microfossils further support the above hypothesis: from 40 cm downwards, apart from reworked Cretaceous planktonic foraminifera, many thecamoebians (unicellulars, living only in fresh-water fast flowing streams) and marine-to-brackish water benthic foraminifera are found (*Nonion*, *Ammonia*, *Elphidium* and *Bulimina*). Autochthonous planktonic foraminifera are absent (W. J. Zachariasse personal communication).

Therefore the sediment of the lower part of the core must have been deposited very close to the continent in a shallow fresh-to-brackish water environment. Cretaceous material must have entered the sediment column through erosion (river, ice-berg rafting); chalk pebbles all have rounded forms. Then, at a certain moment the sediment 'slab' must have slid down, with preservation of stratification and pore water composition. The

thickness of the preserved sediment column must be at least 20 m. Calculations on diffusion can be made, assuming an original chloride concentration in the slide to be 0‰. After some simplification the solution of Fick's 2nd law becomes³⁹:

$$c = C(\operatorname{erfc} x/2\sqrt{Dt}) \quad (1)$$

where c = concentration of Cl in pore water; C = concentration of Cl in the bottom water (560 mmol l⁻¹); $\operatorname{erfc} x/2\sqrt{Dt} = 1 - \operatorname{erf} x/2\sqrt{Dt}$; erf = error function³³; D = effective diffusion coefficient, $3.5(\pm 0.5) \times 10^{-6}$ cm² s⁻¹; t = time (s).

The effective diffusion coefficient (D) was calculated from the porosity and from the diffusion coefficient of chloride in free solution^{17,24-27,40-47}. Porosity was determined by water content (wet weight and dry weight measured), density of the pore water and assuming a grain density of 2.65 g cm⁻³. Since porosity was nearly uniform from 40 to 600 cm (0.46 ± 0.02), it was considered constant in the diffusion calculations. The value of t found in this way (20,000 years) is to be considered as an upper limit, as there are indications that the sediment must have been brackish at the time of deposition. On the basis of exchange reactions (Fig. 4a), microfossils, and preliminary results of sediment analyses, it can be concluded that not more than the top 40–50 cm were deposited 'after the slide'. Known sedimentation rates for this area during the last 100,000 years were about 3 cm per 1,000 yr⁴⁸. As sliding was probably followed by a short period of high sedimentation, an approximate age for the slide will be 12,000–15,000 years.

To explain triggering of the Storegga slide, Bugge⁴⁹ assumed earthquake activity around 13,000 BP at the end of the glacial period. This fits in rather well with our estimates, and could explain the glacial origin of the fresh water which is suggested by our oxygen isotope data. However, it is uncertain whether the above-mentioned slide is related in any way to the Storegga slide.

Using the value of 12,000 to 15,000 years BP as time of the slide, an estimate can be made of the initial salinity of the pore water. Equation 1 can be written

$$\operatorname{erfc} x/2\sqrt{Dt} = c/C \quad (2)$$

If the initial chloride concentration in the sediment is taken to be c_1 , then equation (2) becomes: $\operatorname{erfc} x/2\sqrt{Dt} = (c - c_1)/(C - c_1)$. Writing $c_R = c - c_1$ and $C_R = C - c_1$, one obtains an expression similar to the one in equation (2): $\operatorname{erfc} x/2\sqrt{Dt} = c_R/C_R$. In this way an initial chloride concentration can be calculated. Due to the large possible variation in time, and the uncertainty in D , only a rough estimate of the initial chloride concentration can be given (70–190 mmol l⁻¹), which is equivalent to a salinity of 4–13‰.

Marine slides are not uncommon phenomena⁵⁰⁻⁵²; however, to our knowledge this is the first time, that the occurrence of a marine slide has been demonstrated by pore water data. Moreover, it is shown that during the sliding the structure and pore water composition were preserved ('bedding-plane slide'⁵¹).

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Restoration of Norwegian lakes by reduction in sulphur deposition

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Acid precipitation, causing the acidification of freshwaters and hence damage to fisheries, affects more than 33,000 km² of southern Norway¹. As a first step in dealing with the acidification problem, Norway, Sweden and Finland have proposed that emissions of sulphur in Europe be reduced by 30% by 1993 (ref. 2). Using data from a 1974–75 survey of major-ion chemistry and fish populations in 683 lakes in southern Norway³, we predict here that a 30% reduction in sulphur deposition will restore chemical conditions such that 22% of the lakes now experiencing fisheries problems should be able to support fish.

Our method of prediction derives from empirical relationships between sulphur deposition, water chemistry and fisheries status for several thousand lakes and rivers in Europe and North America^{4,5}. The method takes into account lake-to-lake differences in sensitivity, changes in base cation concentrations, and the buffering of dissolved aluminium at pH < 5.4 and of bicarbonate at pH > 5.4.

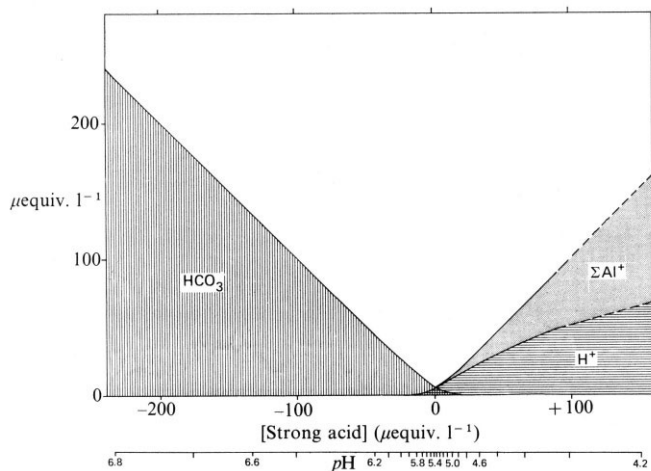


Fig. 1 Concentrations ($\mu\text{equiv. l}^{-1}$) of HCO_3^- , H^+ and ΣAl^{3+} plotted against concentration ($\mu\text{equiv. l}^{-1}$) of strong acid (SA). SA is defined as $\text{H}^+ + \Sigma\text{Al}^{3+} - \text{HCO}_3^-$. Neither ΣAl^{3+} nor HCO_3^- was measured directly in the 700-lake survey. ΣAl^{3+} was obtained from a study of Al speciation over a pH gradient⁸ and is $\Sigma\text{Al}^{3+} = 12.7 - 2.56 \text{ pH}$. HCO_3^- was estimated from the linear regression of $\log \text{HCO}_3^-$ on pH for measurements of 834 samples from lakes and rivers in southern Norway⁴; $\log \text{HCO}_3^- = 5.93 - 1.22 \text{ pH}$ ($r = 0.86$).

For prediction purposes we use the parameter strong acid (SA) which, for lakewater, we operationally define as:

$$\text{SA} = \text{H}^+ + \Sigma\text{Al}^{3+} - \text{HCO}_3^- \quad (1)$$

where H^+ is the hydrogen ion concentration ($\mu\text{equiv. l}^{-1}$) calculated from the measured pH, ΣAl^{3+} is the sum of all positively-charged dissolved Al species ($\mu\text{equiv. l}^{-1}$), and HCO_3^- is the bicarbonate ion concentration ($\mu\text{equiv. l}^{-1}$) (Fig. 1). At pH above ~ 5.4 and below ~ 8.3 , SA is equivalent to the negative value of the bicarbonate alkalinity. Lake waters acidified to $\text{pH} < 5.4$ commonly contain significant concentrations of dissolved aluminium⁷. Hydrogen ions, together with positively-charged Al species, comprise the base-neutralizing capacity in acid waters^{4,6}. Changes in lakewater acidity will be accompanied by changes in ΣAl^{3+} ; the ΣAl^{3+} buffers at $\text{pH} < 5.4$ (refs 7, 8).

In the 1974–75 survey of 683 lakes in southern Norway, 87% of the lakes showed a positive SA ($\text{pH} < 5.4$) and 79% of the lakes had fisheries problems (either barren of fish or sparse populations). The empirical relationship between fish status and SA for these lakes indicates that the probability of a lake having fisheries problems increases from about 0.33 at $\text{SA} = 20 \mu\text{equiv. l}^{-1}$ to 0.95 at $\text{SA} = 30 \mu\text{equiv. l}^{-1}$. This empirical relationship can be used to predict future fish status, given SA.

Previous analyses of the chemical composition of clear, oligotrophic, softwaters in areas in which atmospheric deposition is the only significant pathway for pollutant inputs show that

$$g(\text{Ca}^* + \text{Mg}^*) - \text{SA} = \text{SO}_4^* - b\text{SO}_4^* \quad (2)$$

where the asterisk denotes a non-marine source, $b\text{SO}_4^*$ is the regional background level of non-marine sulphate, g is an empirically derived function, and units are $\mu\text{equiv. l}^{-1}$ (refs 4, 5). The left-hand side gives the sum of loss of alkalinity and increase in base cation concentrations and the right-hand side gives the increase in pollutant sulphate levels. This equation is derived from the ionic balance by subtracting the seawater salt spray contribution, neglecting ions present at low concentrations (NH_4 , NO_3 and organic anions) and incorporating non-marine Na and non-marine K into g (ref. 5). The function g is obtained from regression of $\text{Ca}^* + \text{Mg}^*$ on alkalinity (negative SA) for lakes in areas not receiving significant acid deposition (that is, weighted annual average pH of precipitation > 5)^{4,5}. For lakes in western and northern Norway, Henriksen⁴ finds $g = 0.93(\text{Ca}^* + \text{Mg}^*) - 14$, and this function is used for lakes in acidified southern Norway. We estimate background SO_4^* to be

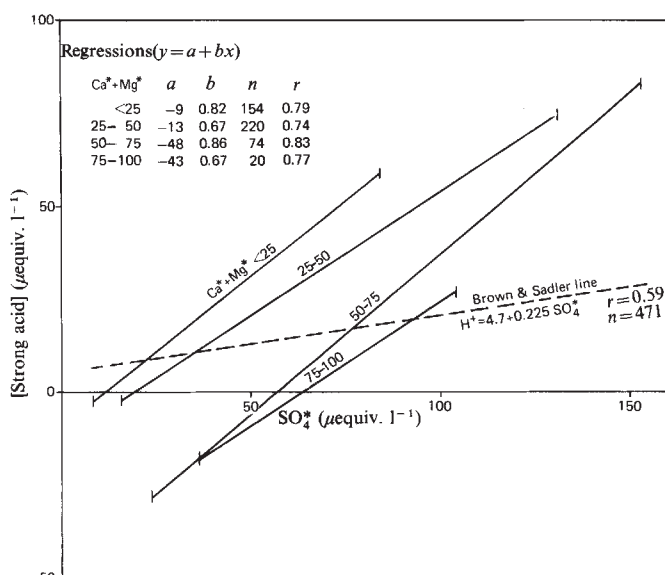


Fig. 2 Least-squares linear regressions for strong acid on non-marine sulphate (SO_4^*) for 471 lakes in southernmost Norway at $> 200 \text{ m}$ elevation above sea level. Lakes are stratified with respect to non-marine Ca + Mg levels. Also shown is Brown and Sadler's regression¹⁰ of H^+ on SO_4^* . Their analysis does not take into account lake sensitivity ($\text{Ca}^* + \text{Mg}^*$ level), ionic Al or HCO_3^- .

$\sim 15 \mu\text{equiv. l}^{-1}$ for southernmost Norway^{4,5}. Background SO_4^* is defined as the natural, pre-industrial level and is presumably due to release of primary sulphur from the terrestrial catchment and to deposition of atmospheric sulphur from natural sources. Of the 683 lakes sampled in southernmost Norway, only five had $\text{SO}_4^* < 15 \mu\text{equiv. l}^{-1}$; these were eliminated from subsequent data treatment.

For the 678 lakes with fish status information and $\text{SO}_4^* > 15 \mu\text{equiv. l}^{-1}$, regression of $[0.93(\text{Ca}^* + \text{Mg}^*) - 14 - \text{SA}]$ on $[\text{SO}_4^* - 15]$ yields $y = 6 + 0.88x$ ($r = 0.85$). The observed concentration of strong acid (and hence pH) is a function of both SO_4^* and $(\text{Ca}^* + \text{Mg}^*)$ in lake water (Fig. 2).

For Norway, nitrate and ammonium can be neglected. Precipitation and dry deposit contain significant but approximately equivalent concentrations of nitrate and ammonium; most lakes in Norway contain $< 10 \mu\text{equiv. l}^{-1}$ of either. The net input of acid or base by nitrogen compounds is thus negligible.

The concentrations of SO_4^* in Norwegian lakes are closely related to the atmospheric loadings of SO_4^* by wet and dry deposition^{4,5}. Least-squares regression of lake SO_4^* on weighted average annual SO_4^* in precipitation gives $y = -19 + 1.9x$ ($r^2 = 0.84$) for 59 lakes in granitic catchments⁹. For 15 groups of lakes in North America the regression yields $y = 14 + 1.9x$ ($r^2 = 0.74$)⁵. The sulphur in acidic deposition increases the SO_4^* concentration in lakes, while the hydrogen ion inputs lead to an increase in strong acid concentrations (loss of alkalinity) or an increase in base cation concentrations, or both. This is simply due to the requirement for electroneutrality.

$$\Delta(\text{Ca}^* + \text{Mg}^*) + \Delta\text{SA} = \Delta\text{SO}_4^* \quad (3)$$

We define the fraction of ΔSO_4^* compensated by a change in $\text{Ca}^* + \text{Mg}^*$ as^{4,5}

$$F = \Delta(\text{Ca}^* + \text{Mg}^*) / \Delta\text{SO}_4^* \quad (4)$$

For oligotrophic softwaters in areas receiving acid precipitation, F is probably in the range 0–0.4 (refs 4, 5). Comparison of modern historical, pre-acidification data indicates $F < 0.4$ (ref. 4). The $\text{Ca}^* + \text{Mg}^*$ and SO_4^* levels in acidified, low-pH lakes suggest even lower F values^{4,5}. For southernmost Norway, $F = 0.2$ appears appropriate⁴.

Change in SA concentration in response to change in SO_4^* is predicted using equations (3) and (4). The predicted fish status

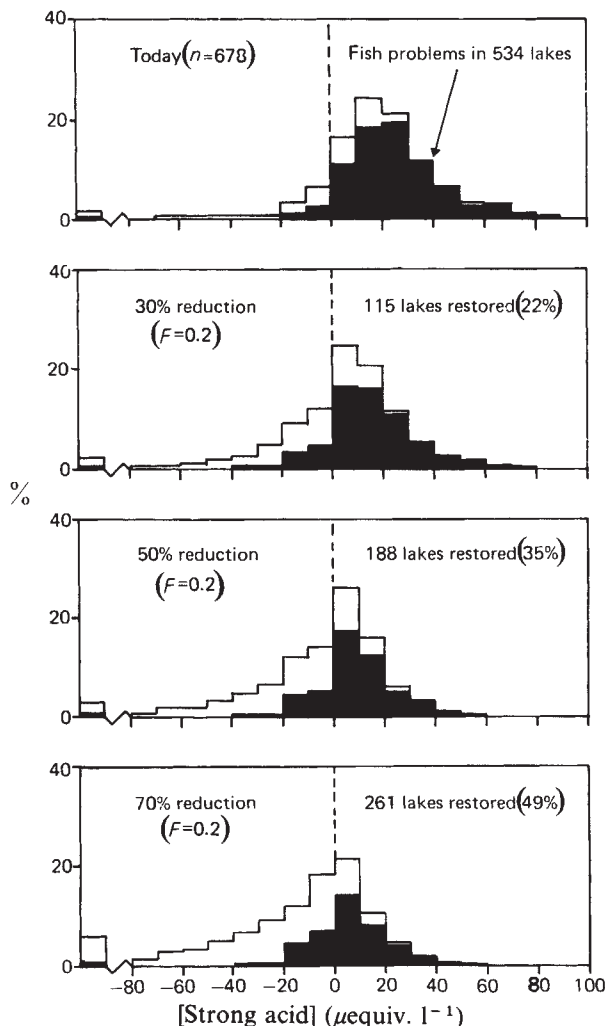


Fig. 3 Frequency distributions of strong acid levels and fisheries status in 678 lakes in southernmost Norway as measured in the 1974–75 survey³, and as predicted after 30%, 50% and 70% reductions in non-marine sulphate concentrations in the lakes. Lakes having fisheries problems (barren or sparse populations) are represented by shaded boxes. A natural background SO_4^{2-} level of $15 \mu\text{equiv. l}^{-1}$ has been subtracted. The fraction of SO_4^{2-} change resulting in change in base cation concentration, F , is set at 0.2.

is then obtained from the empirical relationship between fish status and SA in the lakes. We predict that a 30% reduction in SO_4^{2-} deposition should restore 115 (22%) of the 534 lakes which are having fisheries problems today (Fig. 3). This prediction assumes $F=0.2$, background $\text{SO}_4^{2-} = 15 \mu\text{equiv. l}^{-1}$, and an unchanged relationship between SA and fisheries status. With $F=0$, 27% of the lakes will be restored; with $F=0.4$, 16%. Additional reductions in SO_4^{2-} will restore additional lakes. With $F=0.2$ a 50% reduction will restore 35% of the lakes in southernmost Norway that are experiencing fisheries problems (Fig. 3).

This result contrasts sharply with a previous analysis¹⁰ of the same data, in which it was predicted that a 50% reduction would restore only 11% of the lakes. Brown and Sadler's analysis and subsequent interpretations by Chester¹¹ ignore lake sensitivity ($\text{Ca}^* + \text{Mg}^*$) and buffering of Al and HCO_3^- .

The largest uncertainties in our predictions lie in the estimates of background SO_4^{2-} and F . The choice of bSO_4^{2-} is especially critical for lakes with extremely low concentrations of $\text{Ca}^* + \text{Mg}^*$ and low levels of SO_4^{2-} . Both bSO_4^{2-} and F may differ from lake to lake.

The predictions for lakes in southernmost Norway derive from the 1974–75 survey. Measurements of acid deposition and surface water chemistry in Norway since then reveal no clear long-term trends¹². Although there has been no more recent

regional survey of fisheries, studies at individual sites in southernmost Norway indicate that the fisheries status has continued to deteriorate during the late 1970s¹². This may be a lag effect; as recruitment failure is the most common mode of fish extinction in these lakes, a given lake may not become barren until recruitment fails for several successive years and until the older adult fish die¹³. If the chemical composition of the lakes has not changed since 1974–75 but the fish status has deteriorated, our prediction underestimates the number of lakes that would benefit from a reduction in acid deposition.

Our method does not address the question of rate of response to future changes in loading. Further, we assume that acidification is reversible. Neither of these aspects can be resolved by the data available.

Our prediction that a 30% reduction in sulphur deposition will restore 22% of the lakes in southernmost Norway represents a conservative estimate of the expected improvement in fisheries. Significant reductions will also have a positive effect on salmon and trout populations in rivers and streams, and populations currently experiencing sporadic recruitment failure may be saved.

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Enteric neurogenesis by neural crest-derived branchial arch mesenchymal cells

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We have previously described¹ a monoclonal antibody (E/C8) that recognizes an avian-specific epitope present in a variety of embryonic cells, including some cultured neural crest cells, both central and peripheral neurones *in vivo*, and apparently non-neuronal neural crest-derived² mesenchymal cells of the posterior (third and fourth) branchial arches^{1,3}. The branchial arches are transient embryonic structures that serve as the lateral and ventral walls of the primitive pharynx of vertebrates and are contiguous with the developing gut. We report here that E/C8-positive mesenchymal cells of the arches can develop into neurones spontaneously in culture, or can migrate into aneural guts with which they are co-cultured and form enteric ganglia. In contrast, these cells do not develop into melanocytes—another derivative of the neural crest—in various permissive conditions. These results demonstrate that the mesenchymal cells of the posterior branchial arches are a developmentally restricted population of neural crest-derived cells, and some may serve as precursors for neurones of the enteric nervous system.

The neural crest of vertebrate embryos gives rise to a wide variety of adult cell types, including neurones and glial cells of the peripheral nervous system, pigment cells and various endocrine cells and skeletal elements^{4–7}. The mechanism by which

Fig. 1 E/C8-positive cells in cultures of branchial arch mesenchyme. The third branchial arches from 4-day chicken embryos were dissected, cut into quarters and then placed in culture for 2 days (culture medium and conditions are described in ref. 1). These cultures were then fixed with paraformaldehyde (4%), washed, incubated with a directly labelled horseradish peroxidase (HRP) conjugate of E/C8 (E/C8-HRP) for 1 h in the presence of 0.1% Triton X-100, after which the cultures were washed and peroxidase activity bound to the cells was visualized²⁰. **A** and **C** are phase contrast photomicrographs; **B** and **D** are bright-field photomicrographs to emphasize the HRP-mediated staining. Note the many nerve fibres in **A** and **B** and the perinuclear E/C8 immunoreactivity in some of the non-neuronal cells (arrows) in **C** and **D**. Scale bars, 50 μ m.



Fig. 2 Invasion of the gut and enteric neurogenesis by branchial arch cells. **A** and **C** are CAM co-cultures¹⁵ of 4-day chicken aneural guts with third branchial arch tissue from either a 4-day chicken embryo (**A**) or 3½-day quail embryo (**C**). **B** and **D** are CAM cultures of either 4-day chicken gut alone (**B**) or branchial arch tissue alone (**D**). In **A**, **B** and **D**, cryostat sections (12 μ m) were taken from unfixed cultures, treated with E/C8-HRP for 1 h at room temperature, washed, peroxidase activity visualized²⁰, and then counterstained with toluidine blue. Controls, in which tissue sections were preincubated with unlabelled E/C8 antibody, showed no staining (for other relevant controls, see ref. 1). Note the presence in **A** of E/C8-positive ganglia (arrows) just outside the layer of circular muscle (**M**) as well as nerve fibres (**F**), and the absence of equivalent structures in **B**. In **C**, the CAM co-culture was fixed in Carnoy's fixative, embedded in paraffin, sectioned and then stained using a modified version of the pyronin-methyl green stain for nucleic acids²¹. Chicken (*Gallus gallus*) cells have an evenly distributed stain in the nucleus, whereas quail (*Coturnix coturnix japonica*) cells have a more clumped-appearing chromatin. Cells enclosed by boxes are groups of quail cells in the region of the myenteric and submucosal neuronal plexuses. **C**, cartilage; **F**, fibres; **L**, gut lumen; **M**, smooth muscle layer. Scale bars, 100 μ m.

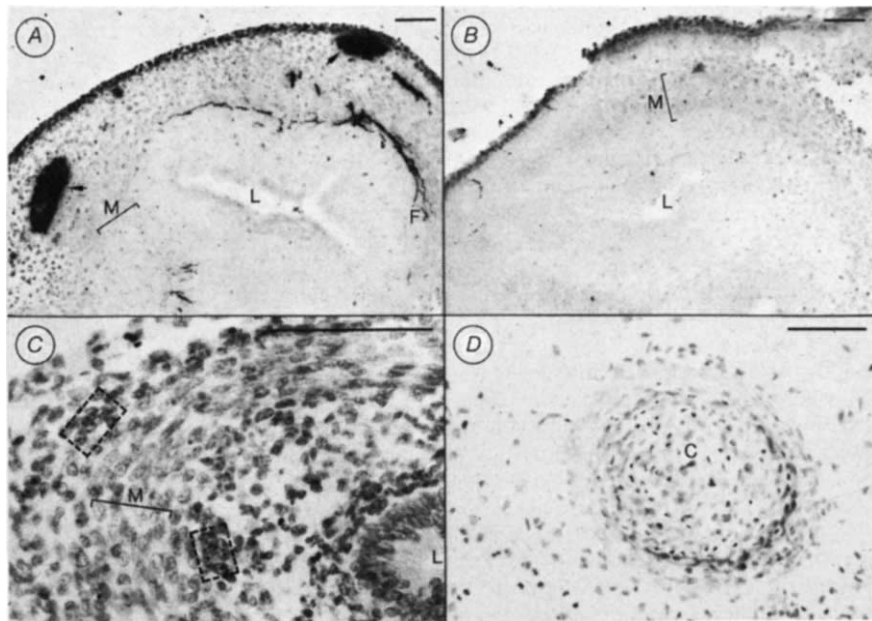
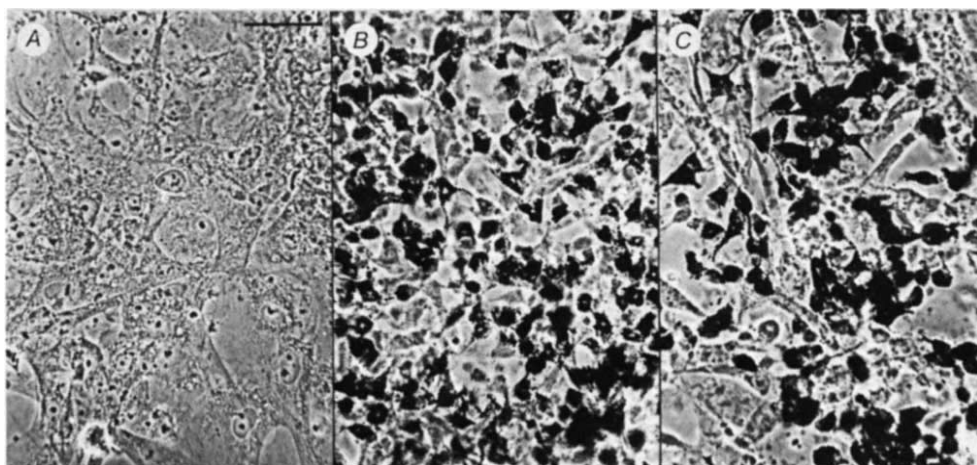


Fig. 3 Melanogenesis by neural crest, but not branchial arch, cells in culture. Phase-contrast photomicrographs of 9-day cultures of either 3½-day quail branchial arch cells (**A**), 2-day quail trunk neural crest cells (**B**), or these two cell types in mixed culture (**C**). Dissection and culture protocols and materials have been published¹⁹. Note the presence of pigmented cells in the neural crest and mixed cultures but not in cultures of branchial arch cells. Scale bar, 50 μ m.



heterogeneity arises among crest cells is still poorly understood, but may involve a cell lineage mechanism^{5,8,9}, similar to that found for various invertebrate nervous tissues¹⁰ and other cell types¹¹. If such lineages exist, one would predict that partially-restricted intermediate cell types appear during development that are able to form some, but not all, of the normal derivatives attributed to their developmental antecedents. The crest-derived cells of the branchial arches seem likely to provide an important test of this prediction.

Because posterior branchial arch cells express the E/C8 epitope and at least one other neuronal trait¹², we tested their ability to develop into neurones in various permissive conditions. In one set of experiments, branchial arches were dissected, grown as explants in a tissue culture environment known to induce neurogenic traits in neural crest cells^{13,14}, and then paraformaldehyde-fixed and processed for E/C8 immunocytochemistry. Figure 1A, B shows the extensive nerve fibre production by some of the cells within these cultured explants, indicating the presence of neurones. As we have previously seen with other types of neuronal cells in culture¹, the cell bodies of these neurones stained only weakly with the E/C8 antibody. Various non-neuronal cells could also be seen within these branchial arch cultures. And while some of these cells exhibited E/C8-mediated staining, typically in a perinuclear location (arrows, Fig. 1C, D), other cells did not.

Since the branchial arch mesenchyme is contiguous with that of the gut, we undertook to test whether these arch cells could develop into enteric ganglionic neurones in an *'in vivo'* system. The third branchial arches from 4-day chicken embryos were excised, cut into quarters and then co-cultured on the chorio-allantoic membrane (CAM)¹⁵ with and without the post-umbilical gut from a 4-day chicken embryo. At this developmental age, this caudal-most portion of the gut is not yet populated with precursors of enteric neurones¹⁶, and therefore provides an ideal permissive environment for enteric neurogenesis. Following a 7-day period on the CAM, co-cultured guts were cryostat-sectioned and then immunocytochemically stained using a horseradish peroxidase-conjugate of the E/C8 antibody (E/C8-HRP). Figure 2A shows the presence of a cluster of E/C8-positive cells and nerve fibres in the region just outside the circular layer of smooth muscle of the gut, where the myenteric plexus neurones normally aggregate. Occasionally, E/C8-positive cells can also be seen in the submucosal plexus or migrating through the muscle layer (not shown). When the gut was cultured alone on the CAM for 7 days, no E/C8-positive cells or fibres were seen (Fig. 2B).

To exclude the possibility that the E/C8-positive cells in the co-cultures were indigenous to the gut, and were somehow induced to express the E/C8-antigen by the arch tissue, quail branchial arches were substituted for chicken in similar co-culture experiments and then sections were examined for the presence of quail cells (branchial arch-derived cells) lying within the chicken tissue. Figure 2C shows the presence of quail cells in the region of the myenteric and submucosal ganglia of the chicken gut. Together, these data strongly suggest that the E/C8-positive cells seen in Fig. 2A are branchial arch cells which have migrated into the gut mesenchyme, aggregated to form ganglia, and have differentiated into neurones (by the criteria of E/C8 expression and the presence of nerve fibres). Similar results have been reported in CAM co-cultures of neural crest cells with portions of the aneural guts of chickens^{17,18}.

Branchial arch tissue which remained following the migration of cells into co-cultured guts was almost entirely devoid of E/C8-immunoreactive cells (data not shown), even though the majority (>90%) of posterior branchial arch cells were E/C8-positive at the start of the co-culture period (G.C. and J.A.W., in preparation). Similar cultures of branchial arch tissue alone contained few, if any, E/C8-positive cells (Fig. 2D). It remains to be shown whether the original E/C8-positive cells migrated away or died (and were replaced by proliferation of the remaining E/C8-negative cells) or whether this phenotypic trait was transiently expressed.

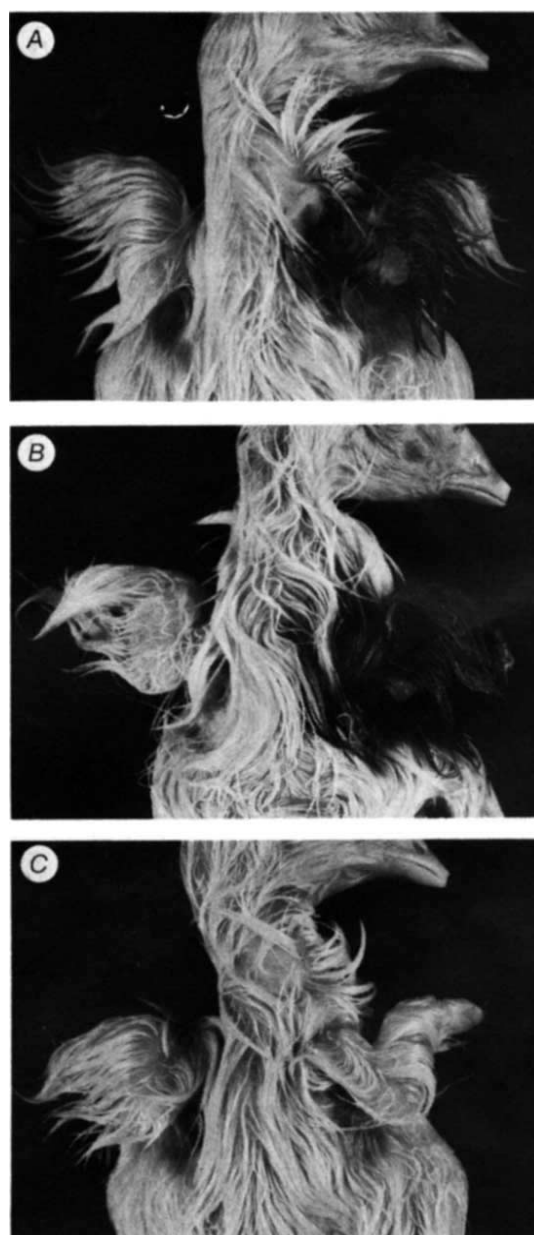


Fig. 4 Melanogenesis by neural crest, but not branchial arch, cells *in vivo*. Two-day quail truncal neural tubes (A), 1½-day quail rhombencephalic neural tubes (B), or the 3rd branchial arches from 3½-day quail embryos (C) were inserted at the base of the right wing bud of 4-day chicken host embryos *in ovo*. The host eggs were then sealed with cellophane tape and allowed to develop for an additional 15 days. Note the pigmented right wings of embryos implanted with sources of neural crest cells, but not with crest-derived branchial arch tissue.

To test their melanogenic potential, branchial arch cells were cultured in conditions known to induce pigment formation in crest cell clusters¹⁹. After 9 days of culture, none of the branchial arch cells had undergone melanogenesis (Fig. 3A), whereas a majority of the crest cells from either trunk (Fig. 3B) or rhombencephalic axial levels (not shown) contained pigment granules (melanosomes). Cultures in which trunk neural crest and branchial arch cells were mixed also contained both pigmented and unpigmented cells (Fig. 3C), thus excluding the possibility that a few cells within the branchial arch somehow inhibited the normal expression of pigmentation.

In another, *in vivo*, test of their melanogenic potential, these same donor tissues (from quail, a pigmented bird) were each implanted into the right wing bud of a 4-day White Leghorn chicken embryo (an unpigmented variety) *in ovo* and then the host embryos were allowed to develop normally over the next

15 days. In Fig. 4, pigmented feathers can be seen in the right wings of embryos implanted with either trunk (A) or rhombencephalic (B) portions of the neural tube, but not with branchial arch tissue (C).

Thus, cells within the mesenchyme of the posterior branchial arches express at least one neuronal trait (E/C8-immunoreactivity) *in vivo* and are capable of becoming neurones in various permissive environments. Although branchial arch cells expressing the E/C8 phenotype may be latent neuroblasts (that is cells irreversibly committed to neurogenesis), the fact that CAM cultures of branchial arches alone do not contain neuronal elements indicates that they require additional environmental cues to undergo ganglionic neurogenesis. However, branchial

arch cells are not simply unrestricted migrating neural crest cells, since they do not share with neural crest cells the capacity to form pigment in permissive conditions in culture and *in vivo*. Instead, these arch cells seem to be an intermediate cell type with a more restricted developmental capability than their neural crest antecedents. The full extent of these developmental restrictions, and the time at which they occur, remain to be determined.

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L1 mono- and polyclonal antibodies modify cell migration in early postnatal mouse cerebellum

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A major event of nervous system development¹ is the migration of granule cell neurones, during the early postnatal development of the cerebellar cortex, from their germinating zone in the external granular layer to their final location in the internal granular layer. During migration, many granule cells are seen in direct cell-surface contact with processes of Bergmann glia, a subclass of astrocytes². In the neurological mutant mouse weaver, however, migration of granule cells is impaired, probably due to a deficit in cell-cell interactions^{3–5}. To gain insight into the cellular and molecular mechanisms involved in granule cell migration, we have used a modification of an *in vitro* assay system, previously described by Moonen *et al.*⁶, which displays migratory behaviour in small tissue explants during several days of suspension culture. The aim of this study was to investigate the process of granule cell migration by using antibodies directed against cell-surface components of developing neural cells. We report here that migration of ³H-thymidine-labelled granule cell neurones can be modified by Fab fragments of both mono- and polyclonal L1 antibodies, but not by Fab fragments of polyclonal antibodies prepared against mouse liver membranes, which also react with cerebellar cell surfaces.

Of the antibodies we have chosen, a polyclonal antibody to a cell-surface antigen, designated L1, seemed to be promising for such investigations, as it was shown to inhibit a Ca²⁺-independent adhesion system of dissociated cells from early postnatal mouse cerebellum and neuroblastoma C1300-derived clones⁷. Moreover, the antigen was detectably expressed on post-mitotic granule cell neurones (Fig. 1f), which constitute the granule cell population that is about to migrate, but not on mitotic or pre-mitotic granule cells^{7,8} (Fig. 2b).

The antigen was expressed in the central nervous system on neurones, but not on glia or in several non-neural tissues tested⁷.

Both monoclonal L1 antibodies and a polyclonal L1 antiserum prepared by immunization with immunoaffinity-purified L1 antigen react on SDS-polyacrylamide gel electrophoresis⁷ to give two bands of apparent molecular weights 140,000 and 200,000. The L1 antigen preparation eluted from a monoclonal antibody column contained the two bands to a degree of 99% purity. Using crude membrane fractions, polyclonal antibody reacted exclusively with these two bands by Western blotting techniques. Absorption of polyclonal L1 antibody with immunopurified L1 antigen led to neutralization of the inhibiting activity of Fab fragments in cell adhesion assays⁷. Certain properties of the antigen were similar to those of other immunologically identified cell-surface glycoproteins, including NS-4 antigen^{9,10}, D2 glycoprotein¹¹, BSP-2 (ref. 12), N-CAM^{13,14} and an antigen common to muscle and brain¹⁵. However, L1 antigen is immunochemically distinct from D2, BSP-2 and N-CAM (C. Goidis, personal communication, and unpublished results).

Migration of ³H-thymidine pulse-labelled granule cells was observed by following the translocation of autoradiographically identifiable cell bodies from the external granular layer (EGL) through the molecular layer (ML) into the internal granular layer (IGL) over a period of 2–3 days after *in vitro* cultivation of cerebellar folium explants from 10-day-old C57BL/6J mice (Fig. 1a, b, Table 1). The temporal sequence of passage from one layer to the other was similar when a thymidine pulse was given *in vivo* or *in vitro* and migration allowed to occur *in vitro* in serum-free conditions (Table 1). Neuronal cell loss was not observed in the present culture conditions and during the limited time period of 3 days. Furthermore, Bergmann glia were oriented in a radial fashion, as can be seen by immunolabelling with antiserum to glial fibrillary acidic protein (Fig. 1d). Expression of L1 antigen was also observed as it is *in vivo*, except that the entire width of the EGL was strongly L1 antigen-positive after explant cultivation for 3 days *in vitro* (Fig. 1e), while only the internal part of the EGL carried detectable L1 antigen levels *in vivo* (Fig. 1f). Migration *in vitro* tended to occur with a similar temporal profile to that *in vivo*, with a slight retardation in entry of granule cells into the molecular layer and an accelerated passage through the molecular layer (Table 1).

Granule cell migration was inhibited when Fab fragments of polyclonal and monoclonal L1 antibodies were added to explant cultures after the thymidine pulse (Figs 1c, 2a, Table 1). This inhibition was significant after 3 days *in vitro* ($P < 0.001$), but was not as visible at 2 days. It is difficult to evaluate whether polyclonal antibodies exert a more pronounced influence on cell migration than do monoclonal antibodies (Table 1, comparison

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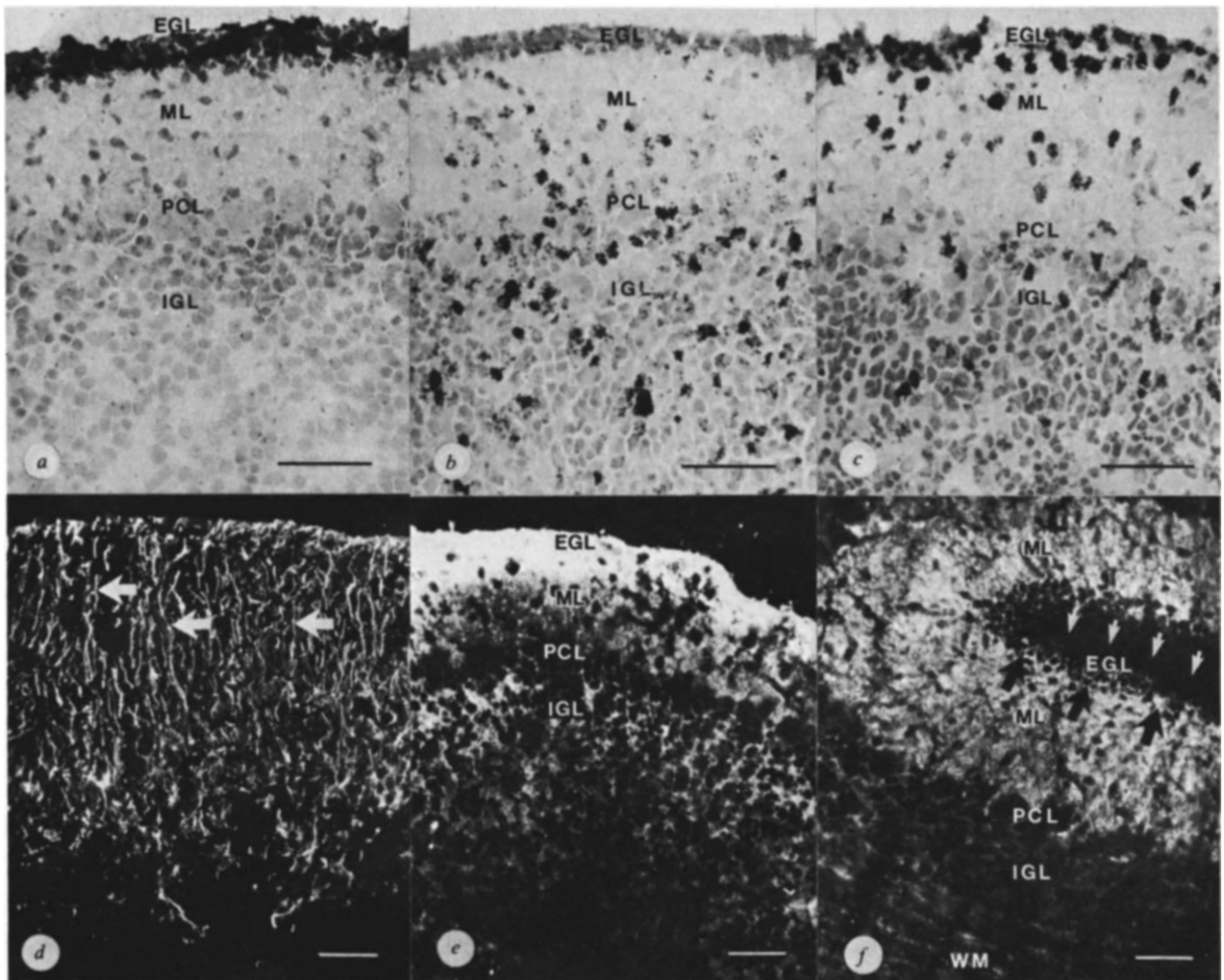


Fig. 1 *a-c*, Distribution of ^3H -thymidine pulse-labelled cells in sections of explants from cerebellar cortex of 10-day-old C57BL/6J mice after various times in culture. *d*, Indirect immunofluorescence on sagittal section of a cerebellar explant after 3 days *in vitro* using antiserum to glial fibrillary acidic protein. Note the histotypic organization of radially oriented Bergmann glial fibres (arrows) in EGL and nascent ML. *e*, Indirect immunofluorescence on sagittal section of cerebellar explant cultivated in medium containing 1 mg ml^{-1} Fab fragments of polyclonal L1 antibody for 3 days *in vitro*. Note that in contrast to the situation *in vivo*, the entire EGL is intensely stained. *f*, Indirect immunofluorescence on sagittal section of 13-day-old mouse cerebellum using polyclonal L1 antibody. Note the lack of staining in the proliferative zone (large arrows) of the EGL. The ML is also intensely stained. Less prominent labelling is seen in the IGL. Fibres are stained in the WM. EGL, external granular layer; ML, molecular layer; PCL, Purkinje cell layer; IGL, internal granular layer; WM, white matter. Scale bars, $50\text{ }\mu\text{m}$. **Methods:** Pieces of folia ($0.6 \times 0.5 \times 1-2\text{ mm}$) were dissected from the cerebellar hemispheres by removal of deep cerebellar nuclei. Explants were then labelled *in vitro* with $1\text{ }\mu\text{Ci ml}^{-1}$ ^3H -thymidine (6.7 Ci mmol^{-1}) for 90 min in defined culture medium¹⁹. After labelling, explants were washed with defined culture medium and maintained (five to seven pieces) in 3 ml culture medium in 25-ml Erlenmeyer flasks on a New Brunswick gyratory shaker at 75 r.p.m., 37°C and 5% CO_2 . Frozen sections in sagittal orientation were cut from these explants at 0 h (after pulse label and washings, before addition of reagents, *a*) and 3 days of maintenance *in vitro* (*b*). For autoradiography, sections were fixed for 5 min at room temperature with 2% glutaraldehyde in phosphate-buffered saline (PBS), pH 7.3, washed several times in PBS, dried, dipped in 0.5% gelatine and coated with Ilford G5 nuclear track emulsion. Autoradiographs were exposed for 12 days at 4°C and processed with Kodak LX24/AL4. Sections were counterstained with haematoxylin-eosin. *c* Shows a section from an explant culture to which Fab fragments of polyclonal L1 antibody (1 mg ml^{-1}) were added once at time 0 h (after pulse label and washings) and which was maintained in Fab fragment-containing culture medium for 3 days. In *d*, indirect immunofluorescence procedures on fresh frozen sections were carried out according to Goridis *et al.*²⁰ In *e*, to visualize the distribution of bound Fab fragments, sections were incubated with the corresponding fluorescent second antibody only. The distribution of polyclonal L1 antibody is identical to the expression of L1 antigen in sections of explants cultured in the absence of antibodies.

of values for IGL and ML). However, it is possible that the small difference seen between monoclonal and polyclonal antibodies reflects differences in actively binding antibody molecules. Total IgG molecules of monoclonal L1 antibody produced a similar retardation to Fab fragments (Table 1). The retardation in granule cell translocation was less pronounced when concentrations of Fab fragments were reduced and fragments added to explants 1 day after the thymidine pulse (not shown). Preincubation of explants with Fab fragments of poly-

clonal L1 antibody (1 mg ml^{-1}) for 15 h before the thymidine pulse resulted in a similar migration pattern to that observed when antibodies were applied directly after the thymidine pulse. Retardation in granule cell migration is not due to death of labelled cells in the presence of antibody, because staining of the explants with propidium iodide on day 3 of culture did not show significant cell death in the external granular or molecular layers. In all cases, the number of thymidine-labelled cells was not detectably affected by L1 antibodies; this was confirmed by

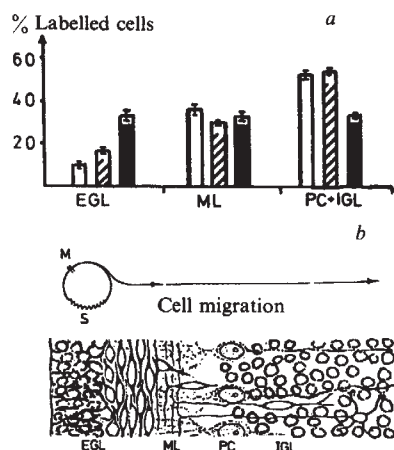


Fig. 2 *a*, Histogram showing the percentage of ³H-thymidine-labelled cells in the three major layers of the developing mouse cerebellum in folium explants after 3 days of maintenance *in vitro*, with and without antibodies. Open bars, culture medium only (control); striped bars, culture medium containing 1 mg ml⁻¹ Fab fragments of polyclonal rabbit anti-liver cell membrane antibody; filled bars, culture medium containing 1 mg ml⁻¹ Fab fragments of polyclonal L1 antibody. *b*, Schematic diagram of the developing cerebellar cortex illustrating stages of increasing granule cell maturation and direction of granule cell migration. For correlation with L1 antigen expression see Fig. 1*e*. (Modified from Willinger and Schachner²¹ and Fujita²².) Culture conditions are described in the legend to Fig. 1.

counting the absolute numbers of ³H-thymidine-labelled cells per unit area of segment comprising all three layers in the histological section.

To verify the specificity of the influence of L1 antibody on granule cell migration, Fab fragments of non-immunized rabbits and rabbits immunized with mouse liver membranes were assayed. These antibodies did not detectably interfere with cell migration (Table 1, Fig. 2*a*). Antibodies to mouse liver membranes bound to the cell surface not only of neurones, but also of glia and fibroblast-like cells in monolayer conditions. This was observed by double immunolabelling techniques using established reference markers¹⁶ for cell type identification. Furthermore, all antibodies applied to the culture medium were detectable by indirect immunofluorescence throughout the entire explant in cryostat sections obtained from explants cultivated for 3 days (see Fig. 1*e* for L1 antibody). The intensity of immunofluorescence staining was slightly less with antibodies to liver membranes than with polyclonal L1 antibody, but in the same range as with monoclonal L1 antibody. Precise quantification, however, is not possible by these methods.

In conclusion, migration of granule cells can be modified *in vitro* by antibodies to L1 antigen. This observation suggests an involvement of one or both components of L1 antigen in cell-cell interactions during or before migration. Possible explanations for this include a modification of adhesive properties among granule cells before migration or interference with Bergmann glia-granule cell neurone interactions by L1 antibodies. It is remarkable that not all small neurones labelled by ³H-thymidine in the external granular layer are prevented from translocation by L1 antibodies. This may be because stellate and basket cells, which assume their final positions in the molecular layer, display a different, L1 antigen-independent translocation mechanism. Furthermore, as Altman has suggested¹⁷, it is possible that not all granule cell neurones move from the external to the internal granular layer by interaction with Bergmann glia. Indeed, the ability of small neurones to move within the cerebellar cortex in the absence of normal cytology and cytoarchitecture has been documented in the case of several neurological mutations of the mouse¹⁸. It is noteworthy that although only polyclonal antibodies inhibit adhesion among neural cells⁷, both monoclonal and polyclonal antibodies affect cell migration. It is conceivable

Table 1 Relative distribution (%) of ³H-thymidine pulse-labelled cells in cerebellar folia of 10-day-old C57BL/6J mice after various times *in vivo* and *in vitro*, with or without antibodies

	95 ± 1.4 2 ± 0.9 3 ± 0.6 n = 1,738 (12)	EGL ML IGL
After thymidine pulse, <i>in vitro</i> or <i>in vivo</i>		
	After 2 days	After 3 days
Pulse label <i>in vivo</i> , maintained <i>in vivo</i>	37 ± 13.8 22 ± 8.5 41 ± 4.6 n = 707 (2)	14 ± 13.3 6 ± 1.3 80 ± 12.0 n = 805 (2)
Pulse label <i>in vivo</i> , maintained <i>in vitro</i>	21 ± 2.3 38 ± 3.0 41 ± 0.6 n = 866 (1)	6 ± 3.8 38 ± 6.2 56 ± 2.4 n = 968 (1)
Pulse label <i>in vitro</i> , maintained <i>in vitro</i>	20 ± 3.2 40 ± 3.8 40 ± 5.9 n = 2,277 (4)	10 ± 1.6 37 ± 2.3 53 ± 2.3 n = 5,353 (8)
Fab fragments of polyclonal L1 antibody (labelled <i>in vitro</i>)	44 ± 11.8 32 ± 6.1 24 ± 6.1 n = 2,085 (5)	33 ± 3.6 33 ± 3.3 34 ± 0.6 n = 3,773 (8)
Fab fragments of monoclonal L1 antibody (labelled <i>in vitro</i>)	30 ± 6.6 46 ± 4.5 24 ± 3.3 n = 2,098 (4)	21 ± 4.7 47 ± 4.1 32 ± 4.1 n = 2,416 (4)
Total IgG of monoclonal L1 antibody (labelled <i>in vitro</i>)	27 ± 6.0 48 ± 1.2 25 ± 4.8 n = 1,740 (3)	18 ± 5.5 45 ± 3.5 37 ± 2.8 n = 1,907 (3)
Fab fragments of anti-liver membrane antibody (labelled <i>in vitro</i>)	22 ± 4.6 33 ± 1.8 45 ± 5.7 n = 1,842 (3)	16 ± 0.8 30 ± 0.7 54 ± 1.5 n = 2,384 (3)
Fab fragments of IgG from non-immune sera (labelled <i>in vitro</i>)	23 ± 4.5 36 ± 0.1 41 ± 4.4 n = 1,101 (2)	16 ± 4.5 33 ± 3.8 51 ± 0.7 n = 1,416 (2)

After ³H-thymidine pulse labelling of cerebellar cells from 10-day-old C57BL/6J mice *in vivo* or *in vitro*, explants were maintained in suspension culture for 2 and 3 days with or without addition of antibodies (1 mg ml⁻¹) after termination of the thymidine pulse. For *in vivo* labelling, animals were injected with 10 µCi per g body weight ³H-thymidine (6.7 Ci mmol⁻¹). Autoradiographs were prepared as described in Fig. 1 legend. Labelled cell bodies having more than 8 grains were counted in fields of standard size. For simplicity, labelled cell bodies in the Purkinje cell layer were included in the counts for IGL. Data represent mean values of percentages and corresponding standard deviations. *n* Represents the total number of cells counted. Values in parentheses indicate the number of independent experiments. First, second and third rows of numbers represent percentage of labelled cells in EGL, ML and IGL, respectively.

that migration, involving a more complex interplay between cells, is more easily perturbed than short-term reaggregation of monodisperse cells. A detailed dissection of the molecular and cellular mechanisms of granule cell migration will depend on the discriminating power and availability of monoclonal antibodies to L1 antigen and its hypothetical receptor(s) and of other antibodies or reagents which inhibit cell migration.

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Motility and mechanosensitivity of macrocilia in the ctenophore *Beroë*

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Mechanical activation of the microtubule sliding mechanism in cilia and flagella^{1–3} by local passive bending has been postulated to be essential for the initiation and propagation of bending waves along the axoneme^{4,5}. In addition, responsiveness of cilia to hydrodynamic forces imposed externally by their neighbours is thought to be responsible for metachronal coordination of ciliary activity^{6–10}, as well as for synchronal beating of component cilia within compound ciliary organelles^{11,12}. Direct tests of the mechanosensitivity of motile cilia are limited, but generally support these views^{7,10,11,13–18}. It remains problematical, however, whether mechanical interaction between cilia operates continuously during both the effective and recovery phases of the asymmetrical beat cycle. Moreover, the directional sensitivity and temporal responsiveness of motile cilia to mechanical stimuli have been explored in only a few cases^{7,10,14}. Finally, the continuous nature of the ciliary beat cycle has hindered investigation of the 'switch point hypothesis' in which doublet sliding is assumed to be activated sequentially on the two halves of the axoneme to produce bends in opposite directions¹⁵. Here we report that macrocilia²⁰ on the ctenophore *Beroë* beat discontinuously with separate effective and recovery strokes, resulting in 'split-cycle' waves of metachronal coordination. This new pattern of ciliary beating is used to investigate the motile responses of cilia to controlled mechanical stimuli during each phase of the beat cycle.

Macrocilia are thick cylindrical organelles found in a dense band around the inner margin of the lips of beroid ctenophores. A single macrocilium is 35–40 μm long and $\sim 5 \mu\text{m}$ in diameter, and consists of $\sim 2,500$ ciliary axonemes cross-linked to one another and surrounded by a common membrane²⁰. Macrocilia beat in a planar ciliary type of pattern with the effective (power) stroke directed away from the mouth. Continuous activity is rarely observed, but when this does occur the cilia beat at 3–5 Hz, with antiplectic metachronal waves sweeping in irregular patterns towards the mouth at 250–300 $\mu\text{m s}^{-1}$.

More typically, macrocilia exhibit an intermittent type of activity termed split-cycle coordination (Fig. 1). At any instant, most of the macrocilia lie at rest at an angle of about 30° to the lip surface with their distal ends overlapping and pointing away from the mouth in the position reached at the end of the effective stroke. Split cycle waves begin at irregular intervals along the aboral edge of the band when a few macrocilia initiate recovery stroke bends towards the mouth. These distally propagated flexions impinge directly on overlying cilia in the oral

direction, lifting them upward and stimulating them into similar activity, which in turn excites their neighbours further orally. A wave of recovery strokes thus travels towards the mouth in a distinct tract, usually 10–20 cilia wide (Fig. 1*a–c*, *a'–c'*). The recovery wavefront, which represents the packed distal ends of the unrolling cilia, advances slowly at a speed of $50 \pm 6.5 \mu\text{m s}^{-1}$ ($n = 12$). The recovery stroke wave usually broadens as it spreads orally, giving the tract a fan-shaped appearance. The macrocilia do not perform an effective stroke immediately, but remain frozen in a sigmoid posture at the end of the recovery stroke for a short time interval. Passage of a recovery wave through a field of resting macrocilia thus leaves a sharply delineated swath of cilia pointing in the opposite, upstream direction.

After a brief time delay, each macrocilium performs an effective stroke and completes the beat cycle before returning to rest. The time interval between the end of the recovery stroke and the onset of the power stroke ranges from $11 \pm 1.5 \text{ s}$ to $13 \pm 1.6 \text{ s}$ ($n = 28$). Although the first cilia to perform an effective stroke are near the aboral end of the tract, a wave of power strokes does not proceed uniformly towards the mouth. Instead, slight variations in the latency between the recovery and effective strokes cause the power stroke to be initiated independently at various locations along the tract, but generally in an aboral–oral sequence. If a macrocilium which makes an effective stroke hits recovery-pointing cilia downstream before they beat spontaneously, these cilia respond by an active power stroke. A wave of effective strokes, usually only a few macrocilia wide, then propagates in an aboral direction for a short distance, ending at cilia further downstream which have already performed their effective stroke (Fig. 1*d*, *e*, *d'*, *e'*). The velocity of the effective stroke waves is $175 \pm 56 \mu\text{m s}^{-1}$ ($n = 17$), or about four times faster than the recovery waves, reflecting the greater angular velocity and closer mechanical coupling between cilia in the effective stroke. After completing the effective stroke, all the macrocilia once again lie at rest pointing away from the mouth, erasing all signs of the original recovery stroke tract (Fig. 1*f*, *f'*). The band of macrocilia is usually covered with many such tracts of split-cycle waves, each acting independently of one another. That this novel pattern of ciliary coordination is not an artefact of excised lip preparations is shown by the presence of split-cycle waves in macrocilia of intact whole baby *Beroë* (1–2 mm long) viewed by Nomarski microscopy.

To investigate the mechanosensitivity of macrocilia further, controlled mechanical stimuli were applied to the cilia at different stages in the beat cycle using fine glass needles operated by a micromanipulator. When resting macrocilia are lifted away from the body surface by a micro-needle pushed in the oral direction, a wave of recovery strokes is initiated which propagates towards the mouth at a velocity of $55 \pm 11 \mu\text{m s}^{-1}$ ($n = 7$) (Fig. 2), similar to the speed of naturally occurring recovery waves. The cilia remain in a sigmoid flexion at the end of the recovery stroke for a short time, and then perform effective strokes as described above. Movement of the micro-needle in the opposite (aboral) direction across the tips of resting macrocilia does not stimulate recovery strokes. Pressing resting macrocilia down against the epithelial surface with a needle stopped the propagation of recovery waves which had begun spontaneously on the aboral side of the needle, further showing that recovery stroke waves are coordinated by viscomechanical coupling between the cilia.

When the tip of a macrocilium temporarily arrested at the end of the recovery stroke is deflected 5–10 μm in the aboral direction by a micro-needle, a single effective stroke may be induced (Fig. 3). The time course of responsiveness to mechanical stimuli was investigated by applying repetitive displacements in the aboral direction to the distal ends of small groups of recovery-pointing cilia, starting 3–5 s after the end of the recovery stroke. In one case, mechanical stimuli delivered at 2-s intervals did not elicit effective strokes until 9 s following the preceding recovery stroke. In another example, a micro-

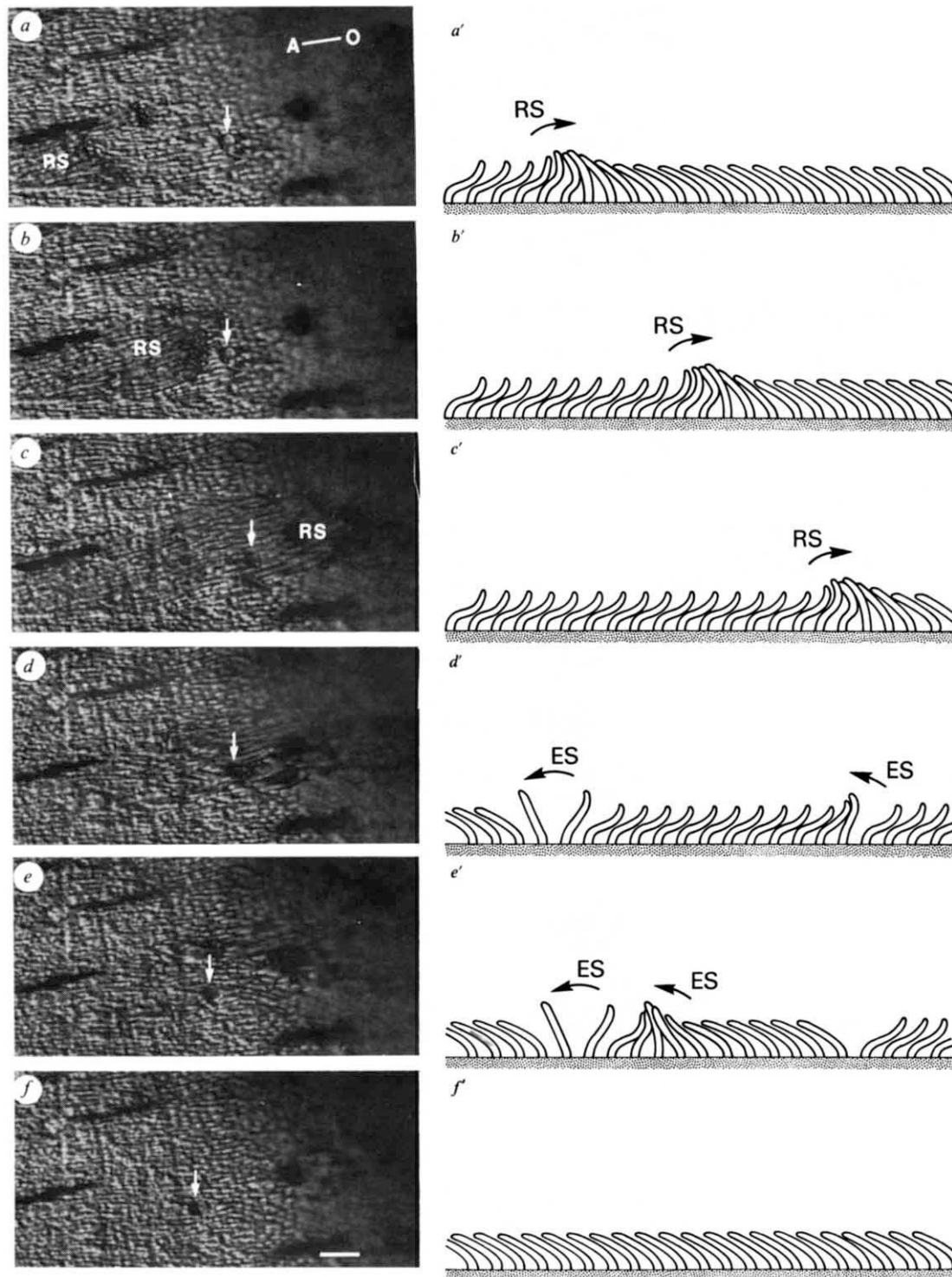


Fig. 1 Face view of field of macrocilia showing sequence of split cycle coordination (Zeiss Nomarski optics, prints from cine film at 2.0-, 3.4-, 8.2-, 0.8- and 5.5-s intervals). *a'-f'*, Diagrams of corresponding stages viewed in profile. *a-c* and *a'-c'*, Recovery stroke wavefront (RS) advancing in an aboral-oral direction (A-O axis), from reader's left to right. Resting macrocilia lie at the end of the effective stroke pointing away from the mouth, and are lifted away from the surface by recovery bends of their neighbours aborally, thereby stimulating a wave of recovery strokes which propagates in the oral direction (RS arrows, *a'-c'*). Macrocilia behind the wavefront remain arrested in a sigmoid flexion at the end of the recovery stroke, leaving a swath of recovery-pointing cilia surrounded by effective-pointing cilia (*a-c*). A particle (arrows, *a-c*) is displaced a short distance in the oral direction by the passing wavefront. *d, d'*, Initiation of the effective stroke after variable time delays at different places along the RS tract, but generally in an aboral-oral sequence. *d*, Most macrocilia on the left side of the tract have completed a power stroke, but those to the right of the particle (arrow) are still doing so. *d'*, Macrocilia on the left are performing an effective stroke (ES arrow) in an aboral-oral sequence; macrocilium on right (ES arrow) initiates an effective stroke prematurely. *e*, Most macrocilia have now performed a power stroke, pushing the particle (arrow) further to the left. *e'*, The premature power stroke by the macrocilium on the right has triggered a wave of effective strokes in macrocilia aboral to it, but this ES wave travels only a short distance to the left before meeting cilia downstream which have already performed a power stroke. *f, f'*, All macrocilia in the tract have completed an effective stroke and lie at rest pointing away from the mouth, erasing all signs of the original recovery stroke wave. The particle (arrow, *f*) has undergone a net displacement to the left (compare with position in *a*) in the direction of the power stroke. Lips of *Beroë cucumis* were excised and mounted in a slide chamber filled with seawater or $MgCl_2$ -seawater (1:1) to relax muscles. Scale bar (*a-f*), 50 μm .

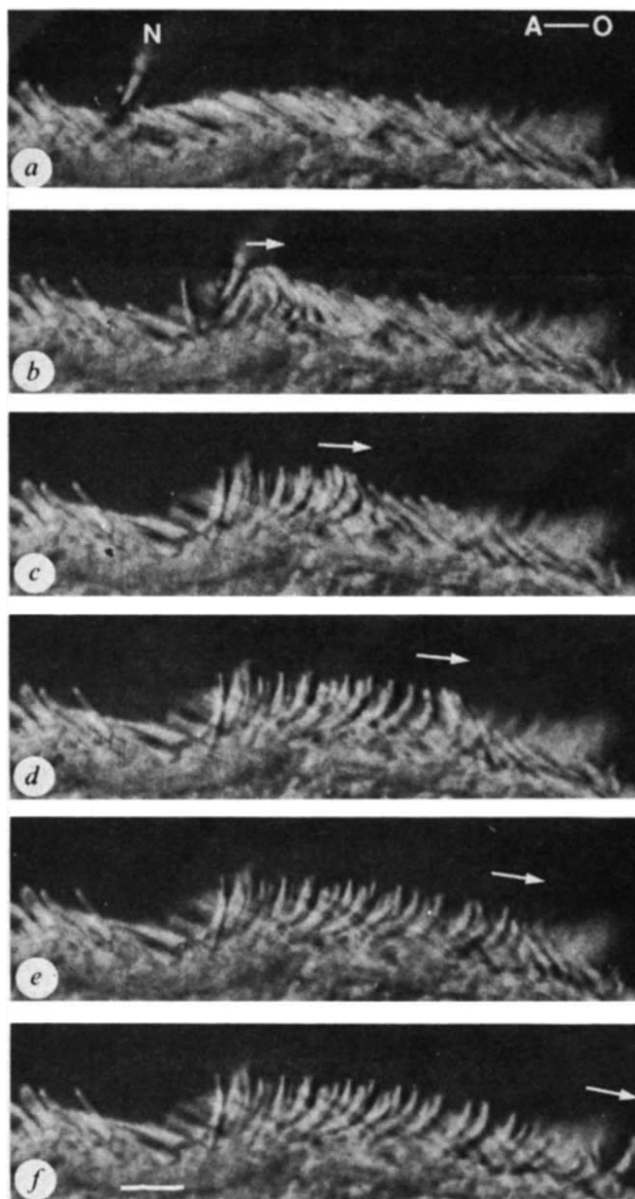


Fig. 2 Mechanical stimulation of a recovery stroke wave in resting macrocilia. Macrocilia are viewed in profile on a thin slice of lip by Nomarski optics. *a*, Before stimulation with a glass needle (N), macrocilia lie over one another pointing in the aboral direction (A-O, aboral-oral axis). *b*, Displacement of several macrocilia to the right (arrow) triggers recovery bends in the oral direction. *c-f*, A wave of recovery strokes propagates towards the mouth (arrows) as cilia in the oral direction are successively pushed upward by their neighbours aborally and stimulated to perform a recovery stroke. Macrocilia remain arrested in a sigmoid posture at the end of the recovery stroke for a brief time before initiating an effective stroke (not shown). Prints from cine film at 0.63-s intervals. Scale bar, 25 μ m.

needle driven by a piezoelectric probe²¹ was used to deliver 10- μ m displacements (0.08 s excursion time) at 1-s intervals to the tips of recovery-pointing macrocilia. No response occurred for 6 s following the recovery stroke; a stimulus at 7 s induced some cilia to perform a power stroke, and the next stimulus at 8 s triggered a few more cilia to do so. The remaining cilia were not activated to perform an effective stroke until 12 s after their recovery stroke.

Therefore, macrocilia arrested at the end of their recovery stroke show a variable period of insensitivity to mechanical stimuli before responding by an active power stroke. This refractory period ends before the cilia perform an effective stroke spontaneously. The motile response of other cilia to mechanical

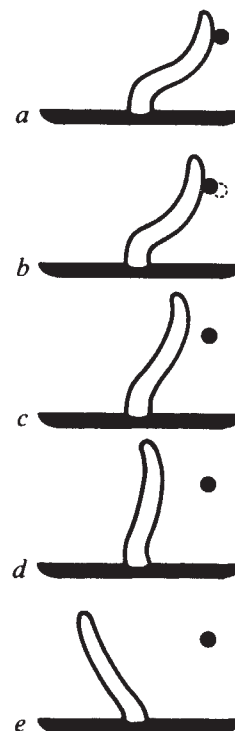


Fig. 3 Mechanical stimulation of the effective stroke in a single recovery-pointing macrocilium. *a*, Micro-needle (black cross-section) is positioned behind the distal end of a macrocilium arrested for the preceding 12.6 s at the end of the recovery stroke (after the refractory period to mechanical stimuli has ended). *b*, The tip of the macrocilium is deflected 5–6 μ m in the aboral direction (to reader's left) by the micro-needle. *c-e*, Initiation of a power stroke in the aboral direction. Tracings from cine film at 0.08-s intervals.

stimulation, excluding arrest responses¹⁵, is usually a complete beat cycle^{7,10,11,14,17}. The effectiveness of the stimulus is a function of its direction in some cases^{10,14}, but not apparently in others¹⁷. In contrast, the immediate response of macrocilia to mechanical stimulation is either an effective stroke or a recovery stroke, but not both. The direction of the active bending response of macrocilia corresponds to the direction of the stimulus. As bending in opposite directions is thought to result from activation of doublet sliding alternately in two halves of the axoneme^{19,22}, our findings indicate that either set of microtubules can be activated independently by mechanical stimuli applied in the direction of force generation.

A unique feature of the beat cycle of macrocilia is the existence of two different arrest positions, one at the end of the effective stroke and the other at the end of the recovery stroke. The resting phase at the end of the effective stroke is of indeterminate duration, lasting until mechanical stimuli trigger a recovery stroke. The pause at the end of the recovery stroke, on the other hand, has a rather constant period which ends spontaneously without external triggering, indicating an intrinsic timing device for activating sliding at the start of the effective stroke. This timing mechanism shows a refractory period during which the mechanosensitivity of the motile elements is reduced or lost. These results support the presence of two distinct switching mechanisms for activation of doublet sliding on opposite sides of the axoneme during a single beat cycle^{19,22} and provide an advantageous system for experimental dissection of the control of microtubule sliding in cilia.

In split-cycle coordination, the recovery stroke waves and effective stroke waves travel in opposite directions, but in the same direction as their respective bending movements (Fig. 1). This provides a clear demonstration of the mechanical coordination of ciliary movement⁶⁻¹². In addition, these observations show that mechanical coupling between responsive cilia can

occur during both the effective and recovery strokes, indicating that metachronism results from continuous mechanical interaction throughout the beat cycle^{6,23}.

Intermittent ciliary activity with resting phases following the end of each effective stroke (but with a continuous beat cycle) is also observed in rabbit tracheal and frog palate cilia, and seems to be an adaptation for mucus and particle transport²⁴. The compounding of several thousand axonemes into a single macrocilium²⁰ would be expected to increase the stiffness and active bending moment of the organelle, allowing *Beroë* to engulf prey larger than itself, or to bite off pieces^{10,25}. The single membrane surrounding each macrocilium, together with the extensive cross-linking of adjacent axonemes, may serve to prevent the organelle from fraying apart during the mechanical stresses associated with prey ingestion.

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Correction of cell-cell communication defect by introduction of a protein kinase into mutant cells

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The cell-to-cell permeability of the junctions of various cultured mammalian cell types depends on the concentration of intracellular cyclic AMP ([cAMP]_i). The permeability rises when the cells are supplied with exogenous cyclic AMP or when their cyclic AMP synthesis is stimulated with cholera toxin or hormones; it falls when [cAMP]_i is lowered by application of serum or due to increase in cell density^{1–5}. The rise and fall in permeability take several hours to develop (the rise is protein synthesis-dependent) and they occur concurrently with the rise and fall in the number of intramembrane particles of the gap junctions^{2–4}, which probably embody the cell-to-cell channels. Is this permeability regulation mediated by phosphorylating protein kinase? In many eukaryotes, the cyclic AMP receptor is a protein kinase^{6–8} consisting of a pair of regulatory subunits and a pair of catalytic subunits. The latter dissociate from the holoenzyme as the cyclic AMP binds to the regulatory subunits and, in this dissociated form, catalyse the phosphorylation of the target⁹. The regulatory subunit occurs in two isoenzyme forms, I and II. The catalytic subunit seems invariant⁹; subunits from different isoenzymes can substitute for each other^{10,11}. We show here that a mutant cell lacking the isoenzyme I is deficient in permeable junctions, and that this junctional defect is corrected when the mutant is supplied with exogenous catalytic subunit.

Chinese hamster ovary cells (wild-type clone 10001, mutant 10260 (I[−]) and mutant 10215 (II[−]), isolated in single-step selection¹², were given by Dr M. Gottesman. Mutant I[−] has no protein kinase type I and has little of type II; mutant II[−] has no protein kinase II and has type I (but this type I has a reduced cyclic AMP sensitivity¹² and the cell's endogenous cyclic AMP concentration is higher than in the wild cell). All three types of cells attached and spread on the culture dishes, establishing close contacts as seen by phase contrast microscopy. Junctional permeability was probed in single-layer cell cultures with carboxyfluorescein (molecular weight (MW) 376) and with glutamic acid labelled with lissamine rhodamine B (LRB-Glu, MW 688). We microinjected these fluorescent tracers into the cells and determined for each individually injected cell the incidence of permeable first-order interfaces, that is, the proportion of fluorescent cells among the cells contiguous to the injected one (Fig. 1). This incidence is a convenient index of changes in junctional permeability, and a particularly sensitive one when the probing molecule is close to detection threshold for junction permeation^{4,5}. All comparisons were done at equal cell densities. For statistical analysis of differences we used the nonparametric Mann-Whitney U method.

Figure 2 gives the mean incidences of permeable interfaces in the three cell types for various density conditions. Mutant I[−] had a significantly smaller incidence than the wild-type cell in all conditions ($P < 0.0005$). The difference between the incidences was most striking with the LRB-Glu probe, as this larger and more negatively charged molecule was close to the detection threshold of junction-permeation in mutant I[−] (Fig. 2c, d; see also the example in Fig. 1). But even the probings with carboxyfluorescein, well above detection threshold, showed a clear difference ($P < 0.0005$) (Fig. 2a, b).

The junctional deficiency and its relation to the enzyme mutation is further demonstrated by clonal analysis of revertants. The I[−] mutation was generally stable¹². However, after growing for several months, a few cultures slowly developed higher base levels of junctional permeability; they seemed to be reverting

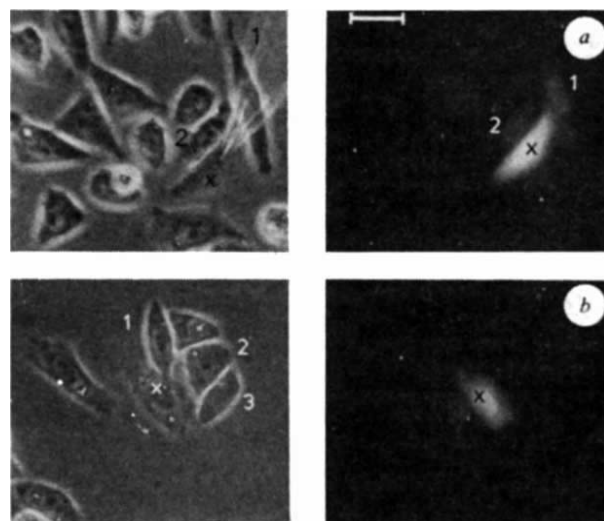


Fig. 1 Junctional probings with LRB-Glu in wild-type (a) and mutant I[−] cultures (b). This probe is close to the detection threshold for junction permeation and therefore is a sensitive indicator of changes in junctional permeability. The injected cell in each cluster is marked by x; the cells contiguous to it are numbered. Left, phase contrast (the injection micropipette is seen in the top part of the cell); right, fluorescence darkfield. Scale bar, 40 μ m. The cells were grown and probed in HAM's F-12 medium with 10% fetal calf serum in plastic dishes (Falcon). They were fed three times weekly, with the last feeding 18 h before experiments, and passaged just before confluency. The last passage was at least 18 h before experiments. For preparation and purification of LRB-Glu, see ref. 20; for steric limits of the cell-cell channel, see ref. 21.

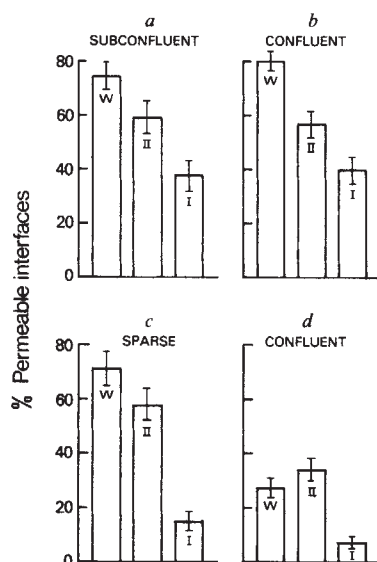


Fig. 2 Incidence (%) of permeable interfaces in the wild-type (W) and mutant cells, I⁻ and II⁻. The incidence of permeable (first-order) interfaces was determined for each individually injected cell. The values shown are the mean incidence $\pm$ s.e., with carboxyfluorescein (a, b) and LRB-Glu (c, d) as the probe, based on 71–247 tests of interfaces. Cell densities (10^4 cells cm^{-2}) were: a, 2–7; c, 1; b, d, 9–20. Statistical confidence level of the differences between the incidences in mutant I⁻ and wild-type is $P < 0.0005$ for both probes and all density conditions. No significant differences were found between mutant II⁻ and wild type ($0.10 > P > 0.05$), except for b ($0.005 > P > 0.001$) (nonparametric Mann-Whitney U test, one-tailed).

to the wild phenotype. Indeed, when we cloned them, these cultures sorted into clones having junctional incidences either like those of the wild type or similar to those of the original I⁻ cell. The difference between the two classes of incidences was in all cases statistically significant ($P < 0.0001$). Moreover, low junctional incidence was invariably found together with the negative protein kinase I trait, and vice versa (Table 1). The correlation between the cell-cell communication and enzyme phenotypes thus also held for genetic backshifts in mutant I⁻.

There was no evidence for a deviation in nonjunctional membrane permeability in this mutant. The fractional rate of fluorescence loss from single cells injected with LRB-Glu, as determined photometrically^{2,4}, was (mean $\pm$ s.e.) $3.1 \pm 0.05 \times 10^{-3} \text{ min}^{-1}$, not different from that of wild-type cells. Clearly, the lower incidence of permeable interfaces in mutant I⁻ represents a lower junctional membrane permeability.

In contrast, mutant II⁻ differed only slightly from the wild-type cell, despite the lower cyclic AMP-sensitivity of its kinase. Only in one of the four probing series of Fig. 2 (b) was the number of permeable interfaces significantly smaller.

Next we asked whether incorporation of the catalytic subunit into mutant I⁻ cells would remedy their junctional deficiency. By means of Kerrick and Krasner's modified 'skinning solution'¹³, we loaded the cells with a purified preparation of the enzyme subunit from bovine skeletal muscle¹⁴—a method already used successfully for introducing another macromolecule into lymphoma cells¹⁵. Among several procedures which we used in trials to render the cells (reversibly) penetrable from the outside by high-molecular weight solutes, we found the treatment with skinning solution simplest. Figure 3 legend details the protocol of the loading experiments. It was based on preliminary trials in which protein A, labelled with fluorescein isothiocyanate (MW 43,000), was substituted for the enzyme subunit (MW 49,000). These trials established that such a molecule gains entry into the cells and that the cells seal after the treatment; and they provided the information necessary for

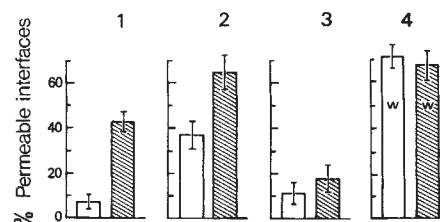


Fig. 3 Results of supplying mutant I⁻ cells with catalytic subunit of cyclic AMP-dependent protein kinase. Values shown are mean incidences of LRB-Glu-permeable cell interfaces in enzyme-loaded (cross-hatched bars, 4–4.5 h after loading) and control cells (open bars). Three different series of experiments were done: series (1) and (2) with active enzyme; series (3) with an enzyme preparation having < 0.001 the activity of the enzyme in series (1) and (2). The mean values of each series are based on 22–38 tests of interfaces. Confidence levels of the differences between incidences in the loaded and control conditions: series (1), $P < 0.002$; series (2), $0.01 > P > 0.005$; series (3), $0.10 > P > 0.05$. The culture in series (2) included revertants (Table 1), therefore the incidence of the controls is higher than in series (1) and (3) and in the experiments of Fig. 2, where the cultures contained I⁻ cells only. Series (4) used wild-type cells (W) and enzyme having the same activity as in (1) and (2) (61 and 42 tests). Series (2) and (4) were sparse cultures of equal densities; (1) and (3) had higher densities (subconfluent).

Methods: Cultures were exposed to skinning solution (at time zero) to which, at 5 min, the catalytic subunit preparation was added ($2.0 \mu\text{M}$ in series (1), $3.24 \mu\text{M}$ in series (2)–(4)). At 25 min, the cultures were rinsed with fresh medium containing catalytic subunit, then incubated in further medium; at 4–4.5 h, junctional permeability was tested. In the controls the catalytic subunit was omitted. Skinning solution (mM): KOH, 81; Na_2ATP , 2; MgO, 3.3; EGTA, 7; imidazole, 84; propionic acid, 67; pH adjusted with propionic acid to 7.0 (free $\text{Ca}^{2+} < 10^{-8} \text{ M}$).

efficient loading. For 10^{-4} M protein A in the medium, almost all the cells of treated cultures became fluorescent and remained so long after the protein A and skinning solution had been rinsed off. At 1 h after the rinsing, protein A presented in the medium no longer entered detectably; and the rate of fluorescence loss from control cells similarly treated with skinning solution (but without protein A) and rinsed, then injected with LRB-Glu, was not different from that of untreated cells.

Figure 3 summarizes the results of the experiments of enzyme loadings in mutant I⁻ cells. In the two series of experiments using active enzyme preparations (series (1) and (2)), the incidence of LRB-Glu-permeable cell interfaces increased significantly above the controls ($P < 0.002$ and < 0.01). (The culture used in series (2) contained revertants; see Table 1. This accounts for the relatively high value of the controls. All other data in Figs 2 and 3 are from I⁻ cultures without evident revertants.)

Series (3), performed with an enzyme preparation that had lost activity on storage, gave no significant increase of transfer. This preparation had less than one-thousandth the activity of the enzyme used in the first two series¹⁶. We found this out only after the three series of experiments had been completed, thus the third series provided an unintended, 'blind' control for the loading method. Moreover, another type of control, in which active enzyme and wild-type cells were used, showed no change in junctional permeability (series (4)). The junctional incidence value of this wild-type culture (68%) may be directly compared with that of series (2) cultures after enzyme loading (65%), which were at the same cell density. (The cultures in series (1) were more dense; the junctional incidences of unloaded wild-type cells at that density were in the range 39–58%.)

We conclude that cyclic AMP-dependent protein kinase seems to play a part in the expression of junctional permeability. Of the two isoenzymes, at least type I seems to be involved at some stage of junctional regulation in culture and seems sufficient for it; this is the only isoenzyme present in the junction-competent

Table 1 Cyclic AMP-dependent protein kinase and communication phenotypes of clones from a reverting culture

Clone	Kinase I (units ml ⁻¹)	Permeable interfaces* (%)
1	392	71 ± 6
2	360	69 ± 5
3	326	72 ± 7
4	403	78 ± 6
5	381	73 ± 6
6	—	7 ± 2
7	—	13 ± 4
8	—	8 ± 3
9	—	11 ± 4
10	—	6 ± 2
11	—	7 ± 2
W	380	72 ± 6
I ⁻	—	12 ± 4

Enzyme assay was done by ³²P transfer from ATP to histone; DEAE chromatography¹⁸. 1 Activity unit = 1 pmol of P per min per mg protein, after subtraction of 'no enzyme' blank value¹⁹. — Denotes undetectable activity (<25 units ml⁻¹). Kinase II ranges from 180 to 260 units ml⁻¹ in all cases (including those in which there was no detectable kinase I activity), except for W, where it is 420. The clones were obtained from the culture of series (2) (Fig. 3). At the bottom of the table are listed the traits of the original wild-type (W) and I⁻ cells (sparse cultures; density as in the clones) as used in all other experiments reported here.

* Incidence of LRB-Glu-permeable interfaces (mean ± s.e.). The confidence level of the difference between the incidences of I⁺ and I⁻ clones was, in all cases, $P < 0.0001$. Means based on 34–88 tests. Sparse cultures.

and cell density-responsive mutant II⁻. A separate role here for the type II isoenzyme, however, is not excluded; we have no mutant available with an enzyme phenotype completely reciprocal to that of mutant II⁻ (in the junction-deficient mutant I⁻, the type II isoenzyme is not abundant). Our results provide no information on the site of phosphorylation, but it is interesting in this connection that the protein of lens gap junction was found to be phosphorylated by a cyclic AMP-dependent protein kinase¹⁷.

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Note added in proof: By electron microscopy of freeze-fractured cell junctions in collaboration with G. Dahl, we have found that the mutant I⁻ has fewer gap-junctional membrane particles than the wild cell.

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High tyrosine kinase activity in normal nonproliferating cells

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Protein phosphorylation at serine and threonine residues has been implicated in the regulation of many cellular processes. More recently, tyrosine residue phosphorylation has been shown to be associated with stimulation of cell proliferation, including viral transformation^{1–7} and stimulation by epidermal growth factors (EGF)^{7–10}, platelet-derived growth factor (PDGF)^{10–12} and other compounds related to cellular growth such as insulin^{13–15} and dimethyl sulphoxide¹⁶. To compare protein kinases and phosphoproteins of normal and leukaemic human haematopoietic cells *in vivo* and *in vitro*, we first have investigated the percentages of phosphoserine, phosphothreonine and phosphotyrosine obtained after hydrolysis of proteins from different blood cell fractions phosphorylated *in vitro*. We report here that phosphotyrosine formed less than 1% of the soluble fractions from polymorphonuclear cells, mononuclear cells (80% circulating lymphocytes, 20% monocytes), blood platelets and red blood cells (not shown). Surprisingly, high percentages of phosphorylated tyrosine were found only in the particulate fractions from non-proliferating anuclear cells, platelets and red blood cells.

A detergent-insoluble particulate fraction was prepared from leukocytes (polymorphonuclear and mononuclear cells) and platelets as described previously¹⁷, and for red blood cells, which possess only minute amounts of detergent-insoluble structures, purified membranes (pink ghosts) were used¹⁸. Protein phosphorylation was carried out for 2 min at 30 °C with [γ -³²P]ATP or [γ -³²P]GTP as phosphoryl donor and either 10 mM MgCl₂ or 2 mM MnCl₂ as divalent cation. Mn²⁺ was used because it has been shown specifically to stimulate tyrosine kinases^{19,20}; concentrations were experimentally determined to give the highest phosphorylation levels (not shown).

Phosphorylated proteins were submitted to acid hydrolysis¹ and analysed for phosphoamino acid content by high voltage electrophoresis¹⁹ and autoradiography. The identity of the different phosphoamino acid spots was confirmed by double-dimension analysis¹ (not shown). Intense phosphotyrosine spots were observed on the autoradiogram only for platelet cytoskeleton and red cell membranes (Fig. 1). In platelet cytoskeleton, phosphotyrosine represented about 20% of the phosphorylated residues with Mg²⁺ and 30–50% with Mn²⁺ as divalent cation. In red cell membranes, phosphotyrosine was mainly detectable with Mn²⁺ (30–50% of the total phosphorylated residues) (Table 1).

Very similar results were obtained with GTP or ATP as phosphoryl donor. Mn²⁺-dependent tyrosine phosphorylation was markedly inhibited by 0.1 M NaCl. The percentages of phosphorylation at tyrosine residues were identical with membranes from either normal (reticulocyte count 1%) or young red blood cells (reticulocyte-rich fraction from a patient with homozygous haemoglobin C disease; reticulocyte count > 15%).

Phosphorylated proteins were analysed by SDS-polyacrylamide gel electrophoresis and some of the gels were treated in a 2 M NaOH solution at 55 °C for 1 h (Fig. 2). The phosphoester linkage to tyrosine is known to be more resistant to alkali than phosphothreonine and phosphoserine^{21–23}.

In mononuclear and polymorphonuclear cells, no alkali-resistant band was observed after phosphorylating with either Mn²⁺ or Mg²⁺ (not shown). In red cell membranes, the pattern depended on whether phosphorylation was carried out on

non-solubilized or detergent-solubilized ghosts. In both cases the 200,000 molecular weight (M_r) spectrin β -subunit was highly phosphorylated in the presence of Mg^{2+} . In contrast, the Mn-dependent phosphorylation of the 93,000- M_r polypeptide group (corresponding to the so-called band 3)²⁴ was only observed with non-solubilized ghosts. The band was alkali-resistant.

In platelet cytoskeleton, many phosphorylated proteins were observed. For some of them, phosphorylation was increased in the presence of $MnCl_2$ (60,000 and 50–55,000 M_r). The most striking finding with platelets was the presence of several clearly alkali-resistant bands: a protein of 60,000 M_r (resolved into two bands in some controls), a broad band of 50,000–55,000 M_r , one band with 45,000 M_r and two bands of <20,000 M_r . The 60,000 M_r band was the most alkali-resistant. After suspension of platelet cytoskeleton in 10% (v/v) detergent buffer (see Fig. 1 legend), centrifugation and autophosphorylation on soluble and insoluble fractions most of the 60,000- M_r alkali-resistant phosphoprotein was found to be strongly associated with the insoluble components (not shown). Some platelet phosphoproteins (120,000, 70,000, 60,000, 50–55,000 and 45,000 M_r) and the red cell phosphorylated β -spectrin (200,000 M_r) and band 3 (93,000 M_r) were eluted from the dried polyacrylamide gels and their phosphoaminoacids analysed.

For platelet cytoskeleton proteins, the percentage of phosphotyrosine was confirmed to be higher with Mn^{2+} than with Mg^{2+} . Some phosphotyrosine was detected in all the bands (Fig. 3), and was the predominant phosphoamino acid residue in the 60,000- and 50–55,000- M_r bands. In red blood cell ghosts, β -spectrin (200,000 M_r) showed no phosphorylation at tyrosine. The 93,000- M_r band, when phosphorylated in the presence of $MnCl_2$, was equally labelled at tyrosine and serine residues while a small amount of phosphotyrosine was observed after Mg -dependent phosphorylation (~9%).

To characterize more precisely the phosphopeptides labelled at tyrosine residues, the bands were cut out from unfixed, undried gel, after rapid autoradiographic exposure. Isoelectric focusing was then carried out in a 8 M urea, 2% NP40, ampholine-polyacrylamide slab gel. For platelets, the 60,000- M_r protein migrated as a narrow band with an apparent pH_i of 4.9–5.0 (not shown). In contrast, pH_i was 5.2 for the 70,000- M_r band. The 50–55,000- M_r group migrated as at least two bands with apparent pH_i s of 5.3–5.5. For red blood cells, the 93,000- M_r band migrated as a broad band in the pH_i range 5.6–5.9.

The 60,000- M_r platelet phosphoprotein was further characterized by partial peptide mapping using V-8 protease (Fig. 4). Several degradative phosphorylated forms were observed, with M_r s of 41,000, 30–33,000, 27,500 and 18,000. All these bands were alkali-resistant and thought to be phosphorylated at tyrosine, which is consistent with the finding that the 60,000 M_r platelet band was labelled almost exclusively at tyrosine.

It was only recently discovered that normal cells could contain very high tyrosine kinase activities. Sefton *et al.*²⁵ reported vinculin to be very slightly phosphorylated on tyrosine in normal chicken fibroblasts (2% of the phosphoaminoacids). Generally, stimulation of phosphorylation at tyrosine residues has been shown in cells stimulated to proliferate^{7–16} and during embryogenesis and development^{26,27}. In all these conditions the percentages of phosphotyrosine remain relatively low. During preparation of this manuscript, however, a membrane-associated tyrosine kinase, phosphorylating band 3, was reported in human erythrocytes²⁸. We report here that, in some conditions, phosphotyrosine represents more than one-third of the phosphoaminoacids incorporated into platelet cytoskeleton and red cell membrane proteins. Due to the relative acidolability of the phosphoryl ester bond at tyrosine²¹, this proportion may even be an underestimate.

It is surprising that such features have not been reported previously. There are two possible explanations for this. First, most of the cytoskeleton phosphorylation experiments have been performed in a so-called 'CSK' buffer containing 0.1 M KCl. We found that monovalent cations were strong inhibitors

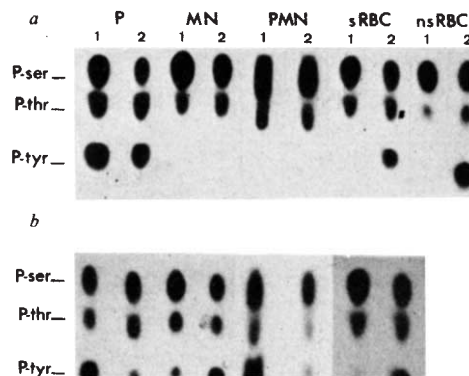


Fig. 1 Electrophoretic phosphoaminoacid analysis in leukocyte and platelet detergent-insoluble particulate fraction, and in red cell membranes. *a*, Phosphorylations were performed in the presence of [γ -³²P]ATP and (1) 10 mM $MgCl_2$; (2) 2 mM $MnCl_2$. P, platelets; MN, mononuclear cells; PMN, polymorphonuclear cells; sRBC, solubilized red blood cell ghosts; nsRBC, nonsolubilized red blood cell ghosts. For red blood cells, weak spots of phosphotyrosine are detectable on the original autoradiograph and not on the photograph. *b*, Phosphorylations were performed in the presence of: (1) [γ -³²P]ATP, 2 mM $MnCl_2$; (2) [γ -³²P]ATP, 2 mM $MnCl_2$ plus 100 mM NaCl; (3) [γ -³²P]GTP, 10 mM $MgCl_2$; (4) [γ -³²P]GTP, 2 mM $MnCl_2$; P platelets; nsRBC, non-solubilized red blood cell ghosts.

Methods: Platelets, polymorphonuclear and mononuclear cells were purified from healthy volunteers' blood, using centrifugation on Ficoll-Paque and Plasmagel sedimentation³⁷. The purity of different cell fractions was checked by direct microscopic examination. Cells were lysed at 4 °C in a 10 mM Tris-phosphate buffer (pH 6.75) containing 1 mM β -mercaptoethanol, 0.1 mM EDTA, 0.2 mM EGTA, 10% (v/v) glycerol, 0.5% (v/v) Triton X-100, 0.5% (v/v) Nonidet P-40, 1% (v/v) Aprotinin, 1 mM phenylmethylsulphonyl fluoride, 0.1 mM diisopropylphosphofluoridate, 0.1 mM pepstatin, 0.1 mM antipain, 0.1 mM chymostatin, 0.01 mM leupeptin, 10 mM ξ -aminocaproic acid and 20 mM benzamidine. Soluble and insoluble fractions were separated by a 30-s centrifugation at 12,000g in an Eppendorf-type table microcentrifuge. The pellet was washed three times in the lysis buffer before being resuspended in a 10 mM Tris-HCl buffer (pH 6.75) containing 0.1 mM β -mercaptoethanol, 0.1 mM EDTA, 0.2 mM EGTA, 5% (v/v) Triton X-100 and 5% (v/v) Nonidet P-40 (100 μ l of this buffer for a pellet corresponding to 1 mg of proteins solubilized in the first lysis buffer). Red blood cells were freed of any contaminating leukocytes and platelets by filtering blood in 0.15 M NaCl on Sigma cell/ α -cellulose (Sigma) columns (1:1 w/w)³⁸. They were then washed in 0.15 M NaCl and lysed in a 10 mM sodium phosphate buffer, pH 7.2. Ghosts were pelleted and washed twice at 27,000g for 30 min before being resuspended in the same buffer as for leukocyte and platelet particulate fraction, with or without detergent. Phosphorylation was carried out at 30 °C in a total volume of 150 μ l containing 100 μ l of the resuspended pellet, 10 mM $MgCl_2$ or 2 mM $MnCl_2$; 5–6 μ M of [γ -³²P]ATP (20–40 Ci mmol⁻¹) were added at times 0 and 1 min; the reaction was stopped at 2 min by the addition of 150 μ l of 30% (w/v) trichloroacetic acid and 150 μ l of cold acetone. The pellet obtained by centrifugation was redissolved in 0.1 M NaOH and reprecipitated twice with trichloroacetic acid (10%, w/v, in final concentration). The final precipitate was washed twice in acetone, dried under reduced pressure and dissolved in 100 μ l of 6 M HCl (Pierce). Acid hydrolysis was performed according to Hunter and Sefton¹ in sealed tubes for 2 h at 110 °C. HCl was then evaporated under N_2 and the hydrolysates were lyophilized twice before being dissolved in 7 μ l of an acetic acid/pyridine H_2O (60:3:937 v/v) buffer, pH 3.2 (ref. 19) containing 0.1 μ M each of phosphoserine (P-ser), phosphothreonine (P-thr) and phosphotyrosine (P-tyr) (Sigma). Electrophoresis was performed in the acetic acid/pyridine buffer on 68 012 CAMAG electrophoresis paper at 3,000 V for 60 min. Standards were visualized by ninhydrin staining and autoradiographs were allowed to develop at -80 °C on Kodak X-Omat AR films using intensifying screens.

of platelet and red cell tyrosine kinases. Similar results were obtained by Richert *et al.*²⁰ with purified Rous sarcoma virus (RSV) p60^{src}. Second, phosphate incorporation into red cell

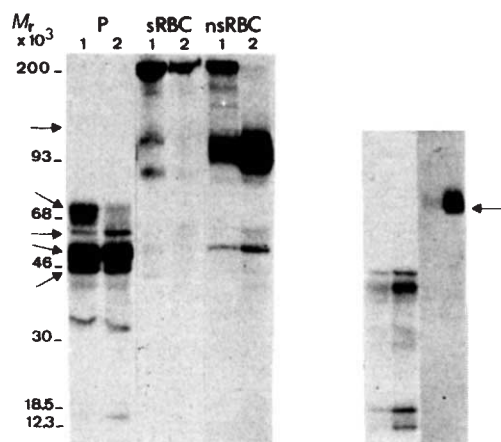


Fig. 2 SDS-polyacrylamide gel electrophoretic analysis of phosphoproteins from platelet cytoskeleton and red blood cell ghosts. Cell extracts phosphorylated in the presence of 10 mM $MgCl_2$ (lanes 1, 3) or 2 mM $MnCl_2$ (lanes 2, 4) were dissociated at 98 °C for 3 min in 0.0625 M Tris-HCl buffer, pH 6.8, containing 10% v/v glycerol, 5% v/v β -mercaptoethanol, 2% w/v SDS and 0.001% w/v bromophenol blue. The samples were then subjected to SDS-polyacrylamide gradient gel electrophoresis according to Laemmli²⁹. Electrophoresed gels were fixed in 10% (v/v) trichloroacetic acid and treated (lanes 3,4) or not (lanes 1, 2) in a 2 M NaOH solution at 55 °C for 1 h, then fixed again. Dried gels were autoradiographed using Kodak X-Omat AR films at -80 °C with intensifying screens. Treated and untreated gels were exposed for the same length of time. P, platelets; sRBC, solubilized red blood cell ghosts (no band was detectable after NaOH treatment (not shown); nsRBC, non-solubilized red blood cell ghosts. ¹⁴C-labelled molecular weight markers were used: myosin (200,000), phosphorylase b (93,000), bovine serum albumin (68,000), ovalbumin (46,000), carbonic anhydrase (30,000), lactoglobulin (18,500) and cytochrome c (12,300). The 120,000, 70,000, 60,000, 50–55,000, 45,000 M_r platelet bands and the 200,000 and 93,000 M_r red blood cell bands are indicated by arrows.

ghosts is very low in the presence of Mn^{2+} compared with Mg^{2+} (ratio ~5). It seems likely that most investigations were carried out with Mg^{2+} as divalent cation, whereas red cell membrane tyrosine kinase is almost exclusively Mn-dependent.

The tyrosine kinases studied here can be compared with previously described systems. PDGF produced by platelets stimulates tyrosine phosphorylation of 175,000- M_r and 130,000- M_r bands from fibroblast membranes¹². Similar bands, which probably correspond to phosphorylated receptor subunits, are observed with EGF²⁹. In addition, EGF stimulates phosphorylation of several other cellular proteins of M_r 34,000 (ref. 7) and 40–42,000 (ref. 30). Clearly, none of these proteins phosphorylated at tyrosine resemble those we observed in platelet cytoskeleton and red cell membranes. Moreover, we found that EGF was unable to increase phosphorylation at tyrosine residues in red cell membranes (not shown).

Platelet 60,000- M_r protein can also be compared with RSV p60^{v-src} and its cellular counterpart p60^{c-src}, which are membrane- and (or) cytoskeleton-linked tyrosine kinases^{31,32} able to autophosphorylate³³. The partial peptide maps of p60^{v-src}

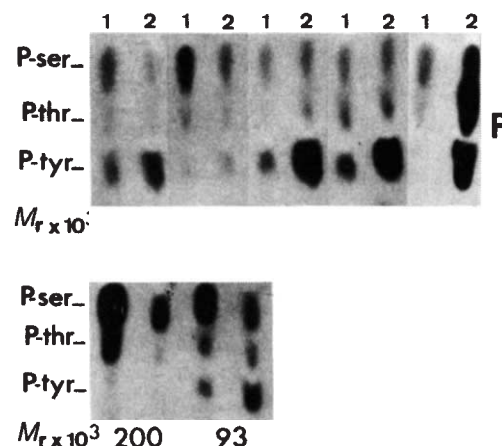


Fig. 3 Phosphoaminoacid analysis of platelet and red blood cell phosphoproteins eluted from polyacrylamide gels. Phosphoprotein bands were eluted from dried polyacrylamide gel according to Blithe *et al.*⁴⁰ or Avruch *et al.*¹⁵, then precipitated by trichloroacetic acid. The precipitates were washed in acetone, dried and hydrolysed before high voltage electrophoresis. Phosphorylations were performed in the presence of 10 mM $MgCl_2$ (1) or 2 mM $MnCl_2$ (2) on platelet cytoskeleton (P) and on non-solubilized red blood cell ghosts (RBC).

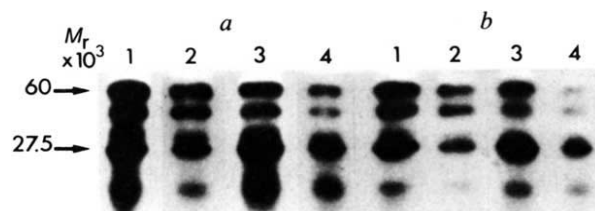


Fig. 4 Partial proteolysis map of the 60,000- M_r platelet phosphoprotein. The 60,000 M_r band was excised after a first SDS-polyacrylamide gradient gel electrophoresis and rerun on a 15% acrylamide gel in the presence of *Staphylococcus aureus* V-8 protease as described by Cleveland *et al.*⁴¹. Platelet extract phosphorylation was performed in the presence of $MgCl_2$ (lanes 1, 3) or $MnCl_2$ (lanes 3, 4). The band was digested with 0.5 μ g (lanes 1, 2) or 1 μ g (lanes 2, 4) of protease. The gel was treated (b) or not (a) with 2 M NaOH at 55 °C for 1 h. The arrows indicate the position of the undigested protein and of the 27,500 M_r polypeptide. Gels a and b were exposed for the same length of time.

and p60^{c-src} are, however, clearly different from that of the platelet 60,000- M_r band³⁴. The only tyrosine-phosphorylated fragment of p60^{src} cleaved by V-8 protease is a 26,000- M_r peptide whereas, for the platelet protein, we found four alkali-resistant bands of M_r 41,000–18,000. In addition, pH_i of the platelet protein (4.9–5.0) differs by at least 1 pH unit from that of p60^{v-src} (~6)³⁵. This argument is, however, less conclusive than that drawn from the peptide maps because the actual pH_i of human p60^{c-src} is not known.

In the red cell membrane, the major phosphotyrosine proteins are located at the level of the 93,000- M_r group (band 3) which

Table 1 Phosphoaminoacid analysis in normal human blood cell particulate fractions

Cells	Mononuclear cells (%)		Polymorphonuclear cells (%)		Platelets (%)		Red blood cells (%)	
	Mg^{2+}	Mn^{2+}	Mg^{2+}	Mn^{2+}	Mg^{2+}	Mn^{2+}	Mg^{2+}	Mn^{2+}
Divalent cation								
Phosphoserine	90	75	82	77	55 ± 8	36.6 ± 3.3	75–87	36–62
Phosphothreonine	9	21	17	21	23.5 ± 8.4	23.4 ± 10.4	11–21	8.5–24
Phosphotyrosine	<2	3	<2	2	21.5 ± 8.0	40 ± 9.5	2–3	30–48

Ninhydrin spots obtained after high voltage electrophoresis of the different cell fractions were excised and counted for radioactivity in 5 ml Biofluor (NEN) scintillation fluid. Each amino acid content is expressed as a percentage of the total phosphoaminoacid radioactivity recovered. The table shows the results of a typical experiment for polymorphonuclear and mononuclear cells, means ± standard deviations from six independent experiments for platelets and extreme values from four independent experiments for red blood cells.

was previously thought to be phosphorylated at serine and threonine by a cyclic AMP-independent protein kinase³⁶. This result confirms that recently reported by Dekowski *et al.*²⁸. Band 3 is a target of tyrosine kinase with nonsolubilized but not with solubilized ghosts. Thus, the major band 3 phosphorylated protein is not the kinase itself and a special steric relationship between the kinase and its substrate could be indispensable to substrate phosphorylation.

The physiological significance of very high tyrosine kinase activities in nonproliferating, terminally differentiated cells

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remains unknown and requires further investigation. However, our finding constitutes a new phenomenon which could be relevant to this type of differentiation. Another obvious implication of these results is that a high phosphotyrosine content, at least after *in vitro* phosphorylation, is not necessarily associated with cancer.

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Stimulation of tyrosine-specific phosphorylation *in vitro* by insulin-like growth factor I

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Several mitogens elicit tyrosine-specific protein kinase activities^{1–7}. Although the physiological significance of this is unclear, the generality of these reactions implies that this may be an inherent feature of growth factor–growth factor receptor interactions. The observed mitogenic properties of the polypeptide insulin-like growth factor I (IGF-I)^{8,9} indicated that it might also stimulate such activity. We report here that IGF-I stimulates a tyrosine-specific protein kinase in a time- and dose-dependent fashion. The close correspondence between an approximate 50% effective dose (ED₅₀) of phosphorylation and an approximate K_d for IGF-I binding leads us to conclude that a high-affinity IGF-I receptor, not the structurally similar insulin receptor¹⁰, is the mediator of IGF-I-stimulated kinase activity. Immunoprecipitation indicates that both the β -subunit of the IGF-I receptor and the β -subunit of the insulin receptor are targets for the IGF-I-stimulated protein kinase.

We partially purified IGF-I receptors by methods published previously¹¹ and performed phosphorylations according to procedures outlined in Fig. 1 legend. When IGF-I receptor preparations were incubated with 2.2×10^{-8} M IGF-I and [γ -³²P]ATP, and subsequently examined by SDS-polyacrylamide gel electrophoresis in reducing conditions (50 mM dithiothreitol, DTT), >90% of the ³²P incorporation into protein was found at a molecular weight (M_r) of 90,000 (90K; Fig. 1A). The labelled bands were excised from the dried gel and their ³²P content measured by Cerenkov radiation. We determined that the IGF-I-stimulated label incorporation is time-dependent and reaches a plateau at 8–12 min (Fig. 1B). A 3–4-fold increase in ³²P content due to IGF-I is evident at these points.

Phosphorylation of the 90K band proceeds in a dose-dependent manner, increasing with increasing occupancy of the IGF-I

receptor (Fig. 2, squares). Half-maximal stimulation of ³²P incorporation into protein is achieved with ~3 nM IGF-I. We can derive an approximate dissociation constant for IGF-I-receptor interaction from the competitive inhibition of ¹²⁵I-IGF-receptor binding by unlabelled IGF-I. These data, represented in Fig. 2 (circles), indicate that the apparent constant for IGF-I dissociation from the IGF-I receptor is ~4 nM. The close correspondence between the approximate ED₅₀ of phosphorylation and the approximate K_d of IGF-I binding suggests that the high-affinity IGF-I receptor, not the structurally similar insulin receptor, is the mediator of IGF-1-stimulated phosphorylations. Further, we have measured the potency of IGF-1 as an inhibitor of insulin binding in these same placental extracts (data not shown) and have verified the conclusion of others¹² that at IGF-1 concentrations < 5×10^{-8} M there is minimal occupancy of the insulin receptor by IGF-1. Our data are consistent with the hypothesis that most of the kinase activity we observe is mediated by the IGF-1 receptor. We cannot rule out the possibility that a second component of IGF-1-stimulated kinase activity might be the result of IGF-1 cross-reactivity with the insulin receptor.

TLC analysis of the amino acids following acid hydrolysis of the receptor revealed that phosphotyrosine is the principle phosphoamino acid produced by IGF-I-stimulated kinase activity (Fig. 3).

To examine the substrate(s) of IGF-I-stimulated protein kinase activity, we used two monoclonal antibodies. The first, antibody IR-3 (Fig. 4B), is highly specific for the IGF-I receptor¹³ and precipitates ³²P-labelled β -subunit of the IGF-I receptor subsequent to IGF-I-stimulated kinase activity. Thus, analogous to the insulin receptor and the epidermal growth factor (EGF) receptor, the IGF-I receptor mediates a ligand-stimulated phosphorylation of itself^{3–5}. In these examples, the kinase activity seems to be an intrinsic property of the receptor^{11,14–16} whereas this has yet to be determined for the IGF-I receptor. Surprisingly, a second antibody, IR-1 (Fig. 4C), that recognizes both the insulin receptor and the IGF-I receptor¹³, exposes an even greater phosphorylation of the insulin receptor as a result of IGF-I-stimulated kinase activity (this is taking into account the amount of phosphorylated IGF-I receptor contained in the antibody IR-1 pellet). The data presented in Fig. 2 indicate that the bulk of this phosphorylation is probably

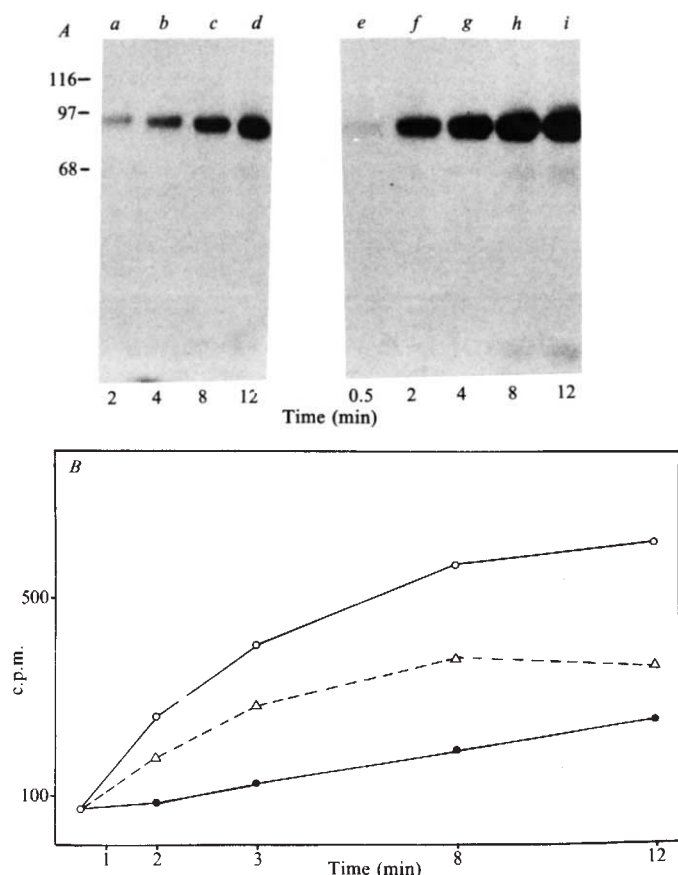


Fig. 1 Time course of ^{32}P incorporation from $[\gamma\text{-}^{32}\text{P}]\text{ATP}$ into a M_r 90K component of human placental membranes. **A**, Detergent-soluble preparation of human placental microsomes enriched in receptors for IGF-I was prepared by the procedure of Shia and Pilch¹¹. The IGF-I receptor (20–40 μg protein²¹) was incubated with (lanes e–i) and without (lanes a–d) IGF-I for 10 min at 23 °C. At this point phosphorylation was initiated by the addition of $[\gamma\text{-}^{32}\text{P}]\text{ATP}$. Final concentrations were 2.2×10^{-8} M IGF-I, 10 mM MgCl_2 , 4 mM MnCl_2 , 50 μM $[\gamma\text{-}^{32}\text{P}]\text{ATP}$ (5–15 μCi), 0.1% Triton X-100, and 50 mM HEPES in a final volume of 100 μl . To stop the reaction at the desired time points (as indicated), an equal volume of electrophoresis buffer (final concentrations 50 mM Tris, 50 mM DTT, 10% glycerol, 1% NaDodSO_4 , pH 6.8) was added and samples were heated at 95 °C for 5 min. Polyacrylamide gel electrophoresis was performed in the presence of NaDodSO_4 on a 7.5% (30:0.8 acrylamide/bisacrylamide) acrylamide gel as described by Laemmli²². The positions of molecular weight markers are indicated on the left (β -galactosidase, M_r 116,000; phosphorylase b, M_r 97,000; bovine serum albumin, M_r 68,000). Autoradiography of the Coomassie blue-stained, dried gel was done at –70 °C with Kodak X-OMAT AR film and Cronex lightening plus enhancing screen. **B**, The M_r 90K region of the dried gel in **A** was excised and the Cerenkov radiation measured in a scintillation counter. The closed circles depict values in the absence of IGF-I, the open circles are values in the presence of IGF-I, and the triangles represent the difference between the two.

due to IGF-I occupancy of the IGF-I receptor. As the number of IGF-I receptors present in our placental extracts was roughly equivalent to the number of insulin receptors, a possible explanation for greater IGF-I-stimulated phosphorylation of the insulin receptor than the IGF-I receptor is that the β -subunit of the insulin receptor is a better substrate for IGF-I-stimulated kinase activity. Another possibility we cannot disregard is that an unknown portion of this phosphorylation is due to IGF-I occupancy of the insulin receptor. Interestingly, Jacobs and co-workers have recently characterized *in vivo* similar IGF-I-stimulated phosphorylation of the insulin receptor¹⁷. There is a growing body of evidence suggesting the relevance of inter-receptor communication. Recently, an insulin-induced increase

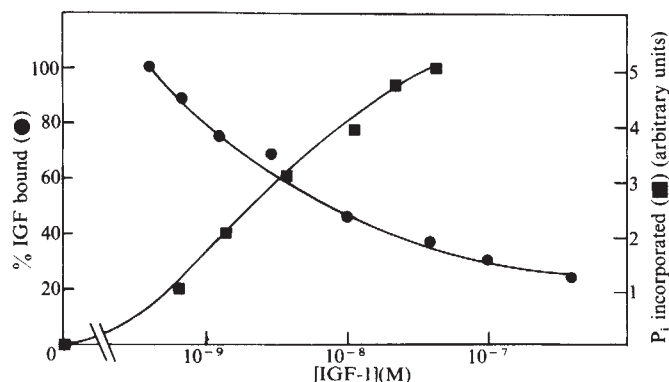


Fig. 2 Dose dependency of IGF-I-mediated ^{32}P incorporation into a M_r 90K component of human placental membranes (■), and competitive inhibition of ^{125}I -IGF-I receptor binding by unlabelled IGF-I (●). Dose dependency of IGF-I phosphorylation: phosphorylations were carried out as described in Fig. 1 legend except that IGF-I concentrations were varied as indicated and all phosphorylations were allowed to proceed for 10 min. Electrophoresis and determination of ^{32}P incorporation followed protocols described in Fig. 1 legend. Competitive inhibition of ^{125}I -IGF-I binding: 20 μl of partially purified IGF-I receptor (8–16 μg) in 50 mM HEPES, 0.1% Triton X-100, 0.02% azide pH 7.6, were added to 30 μl of 50 mM HEPES containing 1 mg ml^{-1} bovine serum albumin, 4×10^{-10} M ^{125}I -IGF-I and concentrations of unlabelled IGF-I to achieve total IGF-I levels as indicated. Incubation of ligand and receptor continued for 30 min at 23 °C and was terminated by the addition of 125 μl of ice-cold 0.05 M NaPO_4 , pH 7.4 containing 1 mg ml^{-1} Cohn fraction II bovine γ -globulins (Sigma), followed by 125 μl of 25% polyethylene glycol, pH 7.6 (ref. 23). Tubes were briefly shaken and placed on ice for 45 min, then samples were centrifuged at 12,000g for 4 min at 4 °C. Supernatants were aspirated and levels of ^{125}I -IGF-I contained in the pellets were determined on an LKB γ -counter.

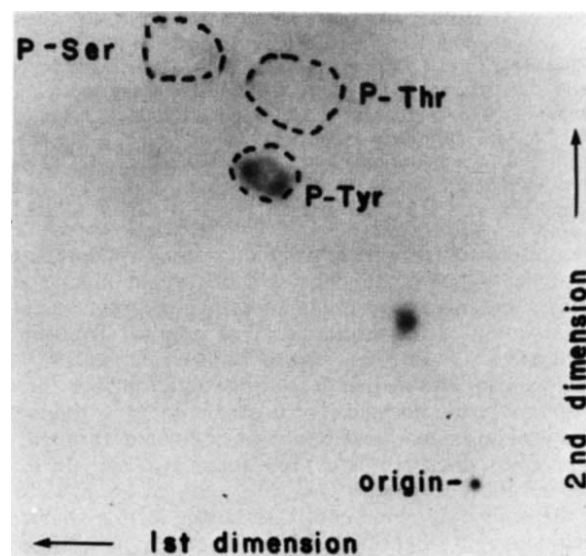


Fig. 3 Two-dimensional thin-layer electrophoresis of phospho-amino acids. Phosphorylation of the IGF-I receptor (see Fig. 1) was stopped by adding 200 μl of unlabelled ATP. This solution was further diluted with 1 ml of 10 mM ammonium bicarbonate. After dialysing overnight against 10 mM ammonium bicarbonate, the phosphorylated receptor was lyophilized. Freeze-dried, phosphorylated receptor was taken up into 0.5 ml of 6M HCl and hydrolysed for 2 h at 110 °C. The hydrolysate was freeze-dried and applied to a 250- μm cellulose TLC plate. Electrophoresis conditions were exactly as described by Hunter and Sefton¹.

in insulin-like growth factor 2 (IGF-II) receptor affinity for IGF-II has been reported to occur in fat cells^{18,19}. Similarly, Bowen-Pope *et al.*²⁰ have observed a platelet-derived growth factor inhibition of EGF binding due to a diminution of EGF receptor affinity for EGF. Documentations of such receptor-

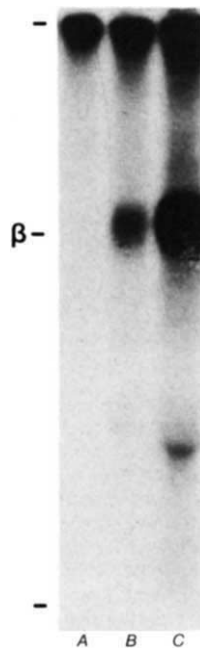


Fig. 4 Immunoprecipitation of IGF-I-stimulated phosphorylation. Phosphorylations were carried out as described in Fig. 1 legend except that ^{32}P incorporation was halted by adding unlabelled ATP to 1 mM. A nonspecific mouse ascites fluid was added to sample A at a 1:200 final dilution. Sample B received IR-3, a monoclonal antibody highly specific for the IGF-I receptor, also at a 1:200 final dilution. Antibody IR-1, which recognizes both the insulin receptor and the IGF-I receptor, was added to sample C at a final dilution of 1:150. These samples were allowed to incubate for 16 h at 4 °C, at which point goat anti-mouse immunoglobulins (Cappel) were added to final dilutions of 1:30 in samples A and B and 1:7 in sample C. The final dilutions of second antibody were optimized for each first antibody. The samples were incubated for 4 h at 4 °C, then 4 ml of 50 mM HEPES, 0.1% Triton X-100, 0.02% azide, pH 7.6 were added to dilute the precipitate and samples were centrifuged at 12,000g for 15 min. Pellets were washed in 4 ml of the same HEPES buffer and finally resuspended in 150 μl of Laemmli sample buffer containing 50 mM DTT, and boiled for 2 min. Electrophoresis was performed as described in Fig. 1 legend. Marks indicate the top and bottom of the gel.

receptor interactions are potentially interesting. The integration of hormonal signals impinging on a cell might involve such interplay. The present example of IGF-I receptor-mediated phosphorylation of the insulin receptor may be significant in this context.

Thus, we have shown that IGF-I, like other growth factors, stimulates a tyrosine-specific protein kinase in a time- and dose-dependent manner. This kinase, mediated through the IGF-I receptor, phosphorylates the β -subunits of both the IGF-I and the insulin receptors.

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Expression of I-A^k class II genes in mouse L cells after DNA-mediated gene transfer

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The I-region of the mouse H-2 complex encodes I-region-associated or Ia antigens which are involved in lymphocyte interactions and in the regulation of immune responsiveness. Two Ia antigens, termed I-A and I-E, have been identified; both are cell-surface integral membrane glycoproteins which consist of two noncovalently linked polypeptide chains designated α (molecular weight (MW) $\sim 34,000$) and β ($\sim 28,000$ MW)¹. Their expression is restricted to B lymphocytes and to antigen-presenting cells such as macrophages. Inside the cell, these $\alpha\beta$ dimers are associated noncovalently with a nonpolymorphic chain of 31,000 MW designated the invariant (Ii) chain². Cloning of 200 kilobases (kb) of the I-region from mice of the H-2^d and H-2^k haplotypes was accomplished recently in our laboratory^{3,4}, and the genes encoding the four polypeptide chains, A α , A β , E α and E β , were identified. We now describe the successful expression of I-A^k molecules in transfected mouse L cells.

Two genomic clones, one coding for the A α and the other one for the A β polypeptide (Fig. 1), were used to transform thymidine-kinase-negative C3H mouse L cells (Ltk⁻ cells)⁵. The recipient Ltk⁻ cells are also of the k haplotype but due to their fibroblastic origin do not express their endogenous Ia genes. Consequently, the appearance of I-A^k molecules following transformation could be monitored with a panel of monoclonal antibodies which react with various epitopes of the I-A^k molecules^{6,7}. We were forced to carry out these gene transfer studies with k haplotype Ia genes because multiple highly specific monoclonal antibodies are available for these gene products, whereas such a panel of specific reagents is currently lacking for their d haplotype counterpart. We have used a two-site sandwich immunoassay which will detect $\alpha\beta$ dimers present both at the cell surface and in the cytoplasm. A crude cell lysate is prepared and I-A^k molecules are specifically immobilized with an anti-I-A^k monoclonal antibody bound to a solid phase. A second, iodine-labelled monoclonal antibody directed against a different determinant of the I-A^k molecule is then used for the detection. As shown in Table 1, uncloned tk⁺ transformants selected in hypoxanthine-aminopterin-thymidine (HAT) medium and transfected with both the A α and A β genes expressed significant levels of I-A^k molecule when compared with C3H (A^k-positive) splenocytes. The tk⁺ cell populations resulting from transfections performed with either the A α or

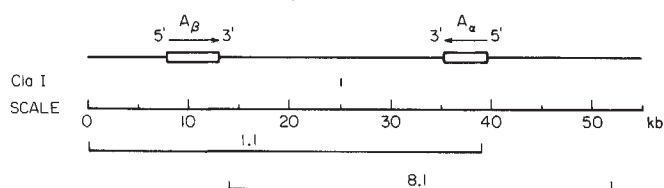
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Table 1 Expression of I-A^k molecules by L cells after DNA-mediated gene transfer

Cells	DNAs used for transfections	Fixation of ¹²⁵ I-labelled antibody (c.p.m.)	
		39J (anti-I-A ^k)	40C (anti-I-E ^k)
CA9.1	pBR322/ASV-2LTR/tk (50 ng)	1,354	1,264
CA8.4	cosH-2 ^k 8.1(A _β ^k) (6 μg) pBR322/ASV-2LTR/tk (50 ng)	1,082	968
CA8.3	cosH-2 ^k 1.1(A _β ^k) (6 μg) pBR322/ASV-2LTR/tk (50 ng)	686	846
CA5.4	cosH-2 ^k 8.1(A _α ^k) (6 μg), cosH-2 ^k 1.1(A _β ^k) (6 μg) pBR322/ASV-2LTR/tk (50 ng)	11,092	920
CA6.1	cosH-2 ^k 8.1(A _α ^k) (6 μg), cosH-2 ^k 1.1(A _β ^k) (6 μg) ptk5 (50 ng)	978	1,468
C3H (H-2 ^k) Spleen cells	None	52,602	1,084
BALB/c (H-2 ^d) Spleen cells	None	1,744	954

Mouse Ltk⁻ cells were transformed with the cosmid clones cosH-2^k8.1 (A_α^k) and cosH-2^k1.1 (A_β^k). Two different plasmids into which the HSV tk gene is inserted were used as selection markers. The plasmid ptk5 contains the BamHI fragment of the tk gene inserted at the BamHI site of pBR322. In addition to the tk gene, pBR322/ASV-2LTR/tk contains two copies of the u3.u5-terminal repeats from the avian sarcoma virus (ASV)⁹. DNAs were linearized before transformation using either HindIII (ptk and pBR322/ASV-2LTR/tk) or ClaI (cosmid clones) (Fig. 1). The amounts of DNA used for each experiment are indicated. No Ltk⁻ carrier DNA was used. Calcium phosphate-DNA precipitates were applied to 1–2 × 10⁶ cells for 20 min, diluted 10-fold with culture medium and the cells were incubated for 4 h at 37 °C. The media was then removed and a glycerol shock (15% glycerol, 3.5 min, 37 °C) was applied to the cells. Medium was then replaced and HAT selection was initiated 48 h later. One month after transformation, tk⁺ cells were tested for the presence of I-A^k molecules using a two-site sandwich immunoassay previously developed by one of us (B.M.) for the analysis of HLA antigens⁸. For this purpose we initially selected several pairs of monoclonal antibodies defining different epitopes of the I-A^k molecules and showing no mutual inhibition of their binding⁶. In the experiments reported in the table, the anti-A^k monoclonal antibody 39B was diluted in phosphate-buffered saline (PBS) to a concentration of 50 μg ml⁻¹ and used to coat the wells of polyvinyl microtitre plates by passive adsorption (2 h at room temperature). Unbound 39B monoclonal antibody molecules were washed out. To saturate the remaining solid-phase binding sites, the wells were then incubated for 30 min with 5% bovine serum albumin (BSA) in PBS. Cells to be analysed were collected in exponential growth phase and lysed with a mixture of 0.5% NP40, 1% BSA, 0.1 mM phenylmethanesulphonyl fluoride, 0.02% sodium azide in PBS (500 μl per 10⁷ cells). After 15 min at 0 °C and centrifugation (100,000g, 1 h), the supernatant was analysed in the two-site sandwich assay. 50 μl of the NP40-cell lysate were applied to each well coated with the monoclonal antibody (1 h at room temperature). After washing out the unbound antigens, wells were incubated for 1 h with either an ¹²⁵I-labelled anti-I-A^k monoclonal antibody (39J) or an ¹²⁵I-labelled anti-I-E^k monoclonal antibody (40C) as a negative control. Finally, unbound radiolabelled probe was eliminated and the radioactivity associated with the solid phase was measured. Results are given in c.p.m. and represent the mean of these experiments. Standard errors of the mean of the triplicates were always less than 5%.

the A_β^k gene alone failed to give positive signals when assayed for A_α^k A_β^k synthesis in our immunoassay. However, RNA blot analyses performed on these latter cell populations (CA8.4, CA8.3) unequivocally demonstrate the presence of either A_α^k or A_β^k mRNA transcripts (data not shown). Comparison of the cell populations CA5.4 and CA6.1 (Table 1) indicates that A^k-positive L cells were only observed in the experiments using both the α and β genes and as a selection marker a herpes simplex virus (HSV) tk harbouring plasmid also containing sequences of the long terminal repeat (LTR) of avian sarcoma virus (ASV)⁹. Whether the ASV-LTR sequences enhance the integration and/or the transcription¹⁰ of the transferred Ia genes is under investigation. Analysis of various clones derived from the original I-A^k-positive cell population indicated the existence of extensive heterogeneity in the levels of expression of the A_α^k A_β^k dimer. Some clones showed barely detectable I-A^k molecules, whereas some others presented levels of I-A^k antigens comparable with those on C3H splenocytes. Most of the

**Fig. 1** Organization of two cosmid clones isolated from AKR liver DNA and containing the A_α^k and A_β^k genes. In addition to the A_β^k gene, cosmid 1.1 contains an incomplete A_α^k gene. Open boxes indicate the Ia genes, arrows give the 5' to 3' direction of transcription. The position of a ClaI recognition sequence is indicated.**Table 2** The allospecificities Ia.1, Ia.2, Ia.17 and Ia.19 are present on the (A_αA_β)^k dimer synthesized in the CA5.4-transformed L cells

Monoclonal antibody fixed on wells	Fixation of ¹²⁵ I-labelled antibody (c.p.m.)			
	39J (anti-Ia.19) CA5.4.4 transformants	C3H splenocytes	10.2.16 (anti-Ia.17) CA5.4.4 transformants	C3H splenocytes
39B (Ia.1)	26,096	21,304	1,804	1,750
39C (Ia.2)	636	600	13,060	24,946
10.2.16 (Ia.17)	22,178	28,960	1,638	1,448
39J (Ia.19)	616	948	49,696	73,472
40B	12,010	16,924	34,408	43,352
10B (Ia.7)	485	506	1,384	1,178

Cell lysates obtained either from CA5.4.4 L cells (I-A^k transformants) or from C3H spleen cells were analysed using the two-site sandwich assay described in Table 1 legend. Each well was incubated with 50 μl of NP40-cell lysate corresponding to the equivalent of 10⁶ cells. The monoclonal antibodies 39B, 39C, 10.2.16 and 39J detect determinants of the I-A^k molecules respectively analogous to the serological specificities Ia.1, Ia.2, Ia.17 and Ia.19. The monoclonal antibody 40B detects a specificity which did not correlate with known conventional Ia specificities⁶. The monoclonal antibody 10B was used as a negative control. It detects an epitope of the I-E^k molecule correlating with the conventional specificity Ia.7. Results are expressed in c.p.m. and represent the mean of triplicate experiments. Standard errors of the mean of the triplicates were always less than 5%.

clones expressed intermediate levels of I-A^k antigens. One clone (CA5.4.4) showing a high level of expression was then selected for further studies. Analysis for over 5 months as well as subcloning experiments indicated that clone CA5.4.4 expressed the I-A^k molecule at a constant high level.

Serological analyses using a panel of 12 monoclonal antibodies defining 4 different allospecificities of the I-A^k molecule demonstrated that the I-A^k molecules expressed by the CA5.4.4 L cells are indistinguishable from those produced by C3H spleen cells. As noted in Table 2, the I-A^k-subregion-encoded specificities Ia.1, Ia.2, Ia.17 and Ia.19 are clearly present on the I-A^k molecules expressed by CA5.4.4-transformed L cells. Because appropriate monoclonal antibodies were not available, the other I-A^k allospecificities Ia.3, Ia.15 and Ia.18 could not be tested on the expressed molecules. To compare the size distributions and the charge heterogeneity of the I-A^k molecules synthesized in normal B cells and in the transformed L cells, I-A^k molecules from C3H splenocytes and from CA5.4.4 L cells were labelled biosynthetically using ³H-phenylalanine, isolated by immunoprecipitation and analysed by two-dimensional gel electrophoresis (Fig. 2). The positions of the A_α^k and A_β^k polypeptides from the CA5.4.4-transformed L cells (Fig. 2a) appeared virtually identical to those isolated from C3H spleen cells (Fig. 2b)¹¹. These data suggest that the size distribution and charge heterogeneity of Ia molecules derived from normal B cells and transformed L cells are very similar, if not identical.

Because the transfected Ia genes are identical to the endogenous Ia genes, it is theoretically possible that the gene transfer merely resulted in the activation of endogenous Ia antigens. There are several reasons for believing that the Ia mRNAs and polypeptides are in fact derived from the exogenous Ia genes. First, it is not clear how the addition of exogenous genes by gene transfer could specifically activate the corresponding endogenous genes. We know of no documented example of

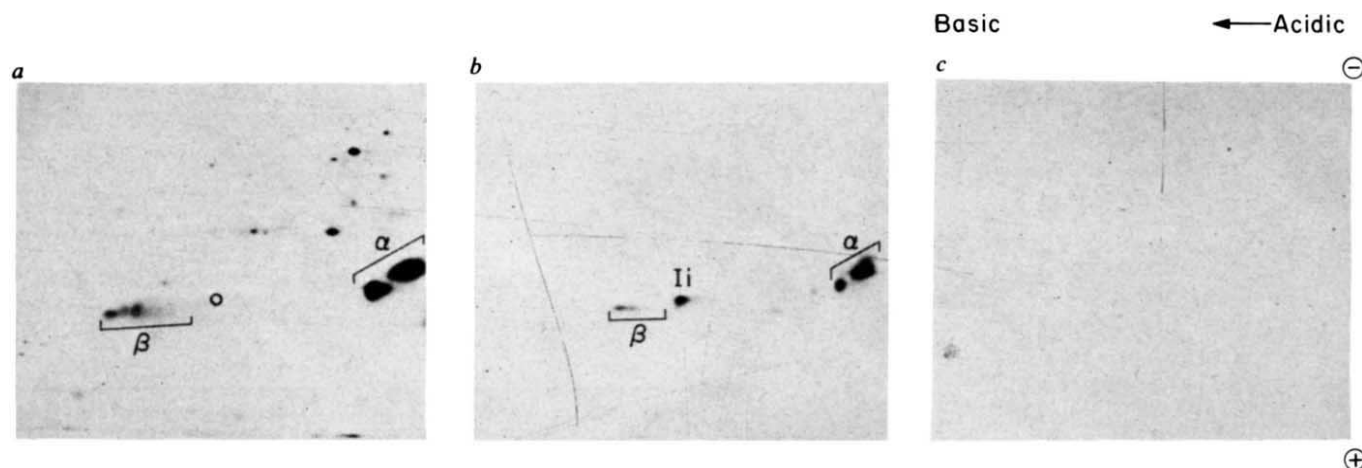


Fig. 2 Fluorographs of two-dimensional gels of I-A^k molecules immunoprecipitated from cell lysates of CA5.4.4 L cells transformed with A_α^k and A_β^k genes (a) C3H spleen lymphocytes (b) and L cells transformed with the tk gene only (c). Cells were biosynthetically radiolabelled with ³H-phenylalanine, immunoprecipitated with the anti-I-A^k 10.2.16 monoclonal antibody and analysed by two-dimensional gel electrophoresis as previously described¹¹. The pH gradient from acidic to basic runs from right to left (first dimension), and the second-dimension SDS gel runs from top to bottom. The gels were prepared for fluorography¹¹, dried and exposed for 2.5 days.

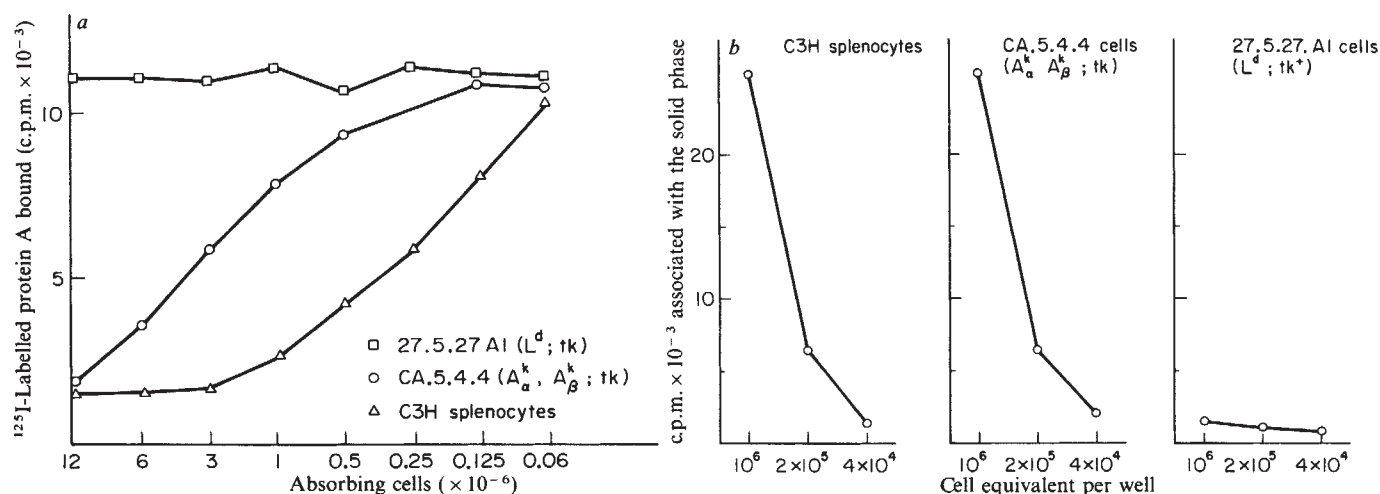


Fig. 3 Comparative analysis of the amount of I-A^k molecules present in CA5.4.4 L cells, I-A^k transformants and C3H spleen cells. **a** Quantitative absorption of monoclonal antibody 39B (anti-I-A^k) with I-A^k-positive C3H spleen cells, CA5.4.4 I-A^k transformants and 27.5.27A1 L^d transformants. **b**, Quantitative analysis of I-A^k molecules present in C3H spleenocytes, I-A^k and L^d transformants using the two-site sandwich immunoassay described in Table 1 legend. In **a**, determined dilution of 39B monoclonal antibody (0.4 μg ml⁻¹) was incubated for 1 h at 4 °C with increasing numbers of either C3H spleen cells (Δ), CA5.4.4 I-A^k transformants (○) or 27.5.27A1 L^d transformants (□). The remaining monoclonal antibody left in the supernatant was tested using an ¹²⁵I-labelled protein A cell-surface binding assay on a constant number (5 × 10⁵) of C3H spleen cells. Results represent the mean of triplicate assays. Control experiments indicated that in contrast to C3H spleenocytes, I-A^k and L^d transformants do not absorb the monoclonal antibody 39G directed to the I-E^k molecules (not shown). In **b**, decreasing concentrations of cell lysates, expressed in cell equivalent per well were incubated in 39B monoclonal antibody-coated wells. The amount of bound I-A^k antigens was evaluated by subsequent incubation with ¹²⁵I-radiolabelled 39J monoclonal antibody. Results represent the mean of three experiments.

such a process. Second, no mRNA or polypeptide products were detected if the cosmid Ia clones were digested with restriction enzymes cutting within the A_α^k and A_β^k genes, before transformation (data not shown). If the exogenous A_α^k and A_β^k genes were somehow activating the expression of their endogenous counterparts, it is not obvious how cleaving the exogenous genes would destroy this effect. Third, the ASV-LTR sequences alone do not activate the expression of the endogenous A_α^k and A_β^k genes (combination CA9.1, Table 1). Fourth, using the E_β^d gene (cosH-2^d24.2), we have also shown that Ia mRNAs of an appropriate size can be expressed in response to gene transfer of Ia genes from mice of a different haplotype (data not shown).

We then analysed the CA5.4.4-transformed L cells for the presence of cell-surface I-A^k molecules using both a trace-binding radioimmunoassay (data not shown) and quantitative absorption analysis. Two important points emerged. First, when compared on a cell basis, the level of I-A^k cell-surface expression by CA5.4.4 L cells was 12 times lower than that of C3H spleenocytes (Fig. 3a). This low level of Ia surface expression

contrasts with the fact that the total amount of I-A^k molecules (surface and cytoplasm) seems to be the same in CA5.4.4 L cells and in C3H spleenocytes (Fig. 3b). Second, some of the monoclonal antibodies used (for example 39J, 39F) will only react with intact Ia molecules containing the fully assembled A_α^kA_β^k molecules¹². Thus, the cytoplasmic I-A^k molecules detected in the cytoplasm are fully assembled. We conclude that large quantities of I-A^k molecules are synthesized and assembled, but that the translocation of these molecules to the cell surface is very inefficient in the transformed L cells.

In contrast to C3H spleen cells, no molecular species migrating at the position of the Ii spot (Fig. 2a, circle) appeared to be associated with the A_α^kA_β^k dimer expressed in the CA5.4.4 L cells. The Ii chain has been implicated in the transport of Ia antigens from the cytoplasm to the cell surface¹³. Consequently, we have used a recently isolated genomic clone for the Ii chain (M.S., unpublished results) to transform Ltk⁻ cells with a mixture of clones for A_α^k, A_β^k, Ii and tk. Analysis of the tk⁺ cell clones derived from these experiments showed that the

expression of the Ii polypeptide in these cells does not lead to a dramatic increase of I-A^k molecules at the cell surface (data not shown). Thus, these preliminary results suggest that the Ii polypeptide is not itself sufficient for transporting Ia molecules to the cell surface and is not required for the assembly of $\alpha\beta$ dimers.

We have shown that the co-transformation of mouse L cells with cosmid clones containing the A _{α} ^k and A _{β} ^k genes results in the expression of A _{α} ^kA _{β} ^k dimers which are indistinguishable from the I-A^k molecules synthesized by C3H spleen cells both serologically and by two-dimensional gel analysis. Taken together with the DNA sequence data on the A _{β} (refs 14–16) and A _{α} genes^{17,18}, the results presented here provide unambiguous evidence that the A _{α} and A _{β} genes of the mouse have been identified correctly. This report constitutes a first step in our long term goal to place Ia genes into biologically relevant cells by gene transfer so that their precise functions can be analysed and perturbed by *in vitro* mutagenesis.

We thank P. Singer and B. Dobberstein for an Ii cDNA clone used for the isolation of the Ii genomic clone, M. Cappechi for the pBR322/ASV-2LR/tk and E. Kraig for thoughtful comments on the manuscript. B.M. was supported by CNRS and by a fellowship from Ministère de la Recherche et de la Technologie (France). M.S. was supported by a Senior Lievre Fellowship from the California Division of the American Cancer Society. This research was supported by NIH grant AI 19624. *Note added in proof:* After submission of this paper for publication, we have been able to achieve a high level of expression of I-A^k molecules at the cell surface using a different subline of mouse Ltk⁻ cells.

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Transcriptional activation of immunoglobulin α heavy-chain genes by translocation of the c-myc oncogene

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Our previous studies with the mouse myeloma MOPC 315 (IgA, λ 2) cell line, using myeloma mutants that had deleted the productive α heavy (H)-chain gene, had shown that the excluded α constant-region (C _{α}) allele in these cells is transcriptionally active¹. Recent reports from several laboratories have demonstrated that in many BALB/c mouse myelomas, including MOPC 315, the DNA segment encoding the c-myc oncogene is translocated to a C _{α} allele^{2–4}. The mRNA coding strand of DNA for the c-myc gene is on the opposite strand

from the immunoglobulin gene in this locus^{2,3,5}. We have now investigated the relationship between the c-myc translocation and transcriptional activity of excluded C _{α} alleles in IgA- and IgG-producing mouse myeloma lines. We report here that c-myc–C _{α} recombination events correlate with demethylation and transcription of C _{α} genes. Several novel C _{α} RNA species are produced, which are transcribed from the immunoglobulin-gene sense strand. The larger C _{α} RNAs appear to contain c-myc sequences. Thus the anti-sense strand of the c-myc gene provides a promoter for transcription of the C _{α} gene. This result suggests that in other transformed cells with a c-myc-immunoglobulin gene translocation, including many Burkitt's lymphomas, activation of the adjacent immunoglobulin gene would occur.

Dot-blot analysis of cytoplasmic RNA was used as an initial test for C _{α} -gene transcription in three IgG-producing myeloma lines. Two of the cell lines, MPC 11 (IgG2b) and MOPC 21 (IgG1), contain C _{α} genes in the germ-line configuration, while one of the C _{α} alleles in HOPC 1 (IgG2a) is rearranged to c-myc (ref. 6 and M.D., unpublished observations). RNA from MOPC 21 and MPC 11 gave no hybridization to a C _{α} cDNA probe, while RNA from HOPC 1 was positive (data not shown). This result was confirmed and extended by characterizing cytoplasmic mRNA by RNA blot analysis (Fig. 1a). HOPC 1 RNA includes several major RNA species that hybridize to a C _{α} probe, as well as larger species present in smaller amounts. The multiplicity of bands is similar to that which we have observed with MOPC 315 variant lines¹, as shown for V-1 in Fig. 1e ('Cy'). The excluded C _{α} allele in MOPC 315 wild-type and variant lines is rearranged to the c-myc oncogene (ref. 2; Fig. 2c). The MPC 11 and MOPC 21 RNAs again show no hybridization to the C _{α} cDNA. The integrity of these RNA preparations was confirmed by translation in a cell-free system (data not shown).

As another monitor of transcriptional activity, the extent of methylation of the C _{α} alleles in the various lines was analysed using the isoschizomeric enzyme pair *Hpa*II and *Msp*I. The digestion of MOPC 315 wild-type and V-1 cell DNA with *Hpa*II indicates that there is no significant methylation of the C _{α} region in either the expressed or excluded allele (Fig. 3a). In contrast, the ~17-kilobase (kb) *Bam*HI fragments of the germ-line C _{α} gene in MOPC 21 and MPC 11 are highly methylated (Fig. 3b).

In HOPC 1, one C _{α} allele (6.6-kb fragment) is methylated and the other (5.9-kb fragment) is hypomethylated (Fig. 3c). It is the 5.9-kb C _{α} allele which has rearranged to the c-myc gene (Fig. 3d). The second c-myc hybridization band is in the position of an unrearranged c-myc gene². Figure 2d shows the map of this locus in HOPC 1; the recombination event has occurred at a different site than in MOPC 315 (Fig. 2c). Thus, C _{α} genes in the germ-line configuration are highly methylated and not transcriptionally active (that is, MOPC 21 and MPC 11). Translocation of c-myc to C _{α} is associated with demethylation of the C _{α} component of this locus and expression of C _{α} RNAs.

With the isolation of a genomic clone of this locus from MOPC 315 (Fig. 2c), a study of the structure of the C _{α} RNAs from this line was initiated. The C _{α} gene element is ~1.5 kb long. Thus the size of the largest C _{α} RNAs in V-1 cells (7.5 and 6.5 kb, Fig. 1e) indicates that transcription of this locus includes DNA sequences located far from the α constant region. The DNA downstream from C _{α} includes sequences for the membrane exon of the α H chain (M _{α} ; see Fig. 2a) and is present in the germ-line and MOPC-315-excluded C _{α} allele (M.D., unpublished observations). No hybridization to an M _{α} probe was detected with V-1 RNA (Fig. 1b). Wild-type MOPC 315 RNA, tested as a control, gave two bands of hybridization at the expected mobilities for membrane-associated RNAs⁷. Thus the cytoplasmic RNA transcripts from the excluded C _{α} locus in MOPC 315 do not contain sequences 3' to the C_H3 domain. Therefore, the possibility that these large transcripts include sequences from the switch region and the c-myc locus was tested.

Cytoplasmic RNA from MOPC 315 V-1 cells was hybridized to a probe containing α switch-region sequences (probe S _{α} , Fig.

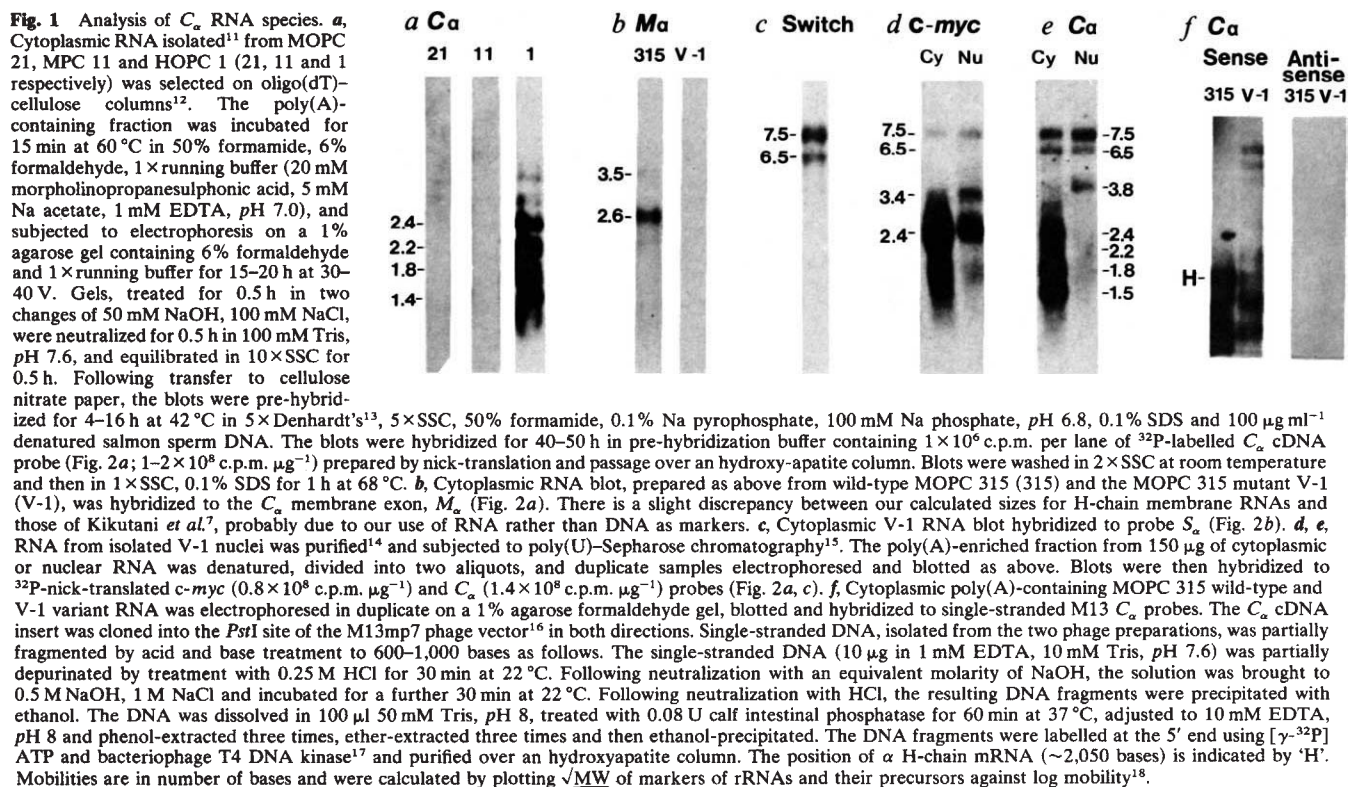
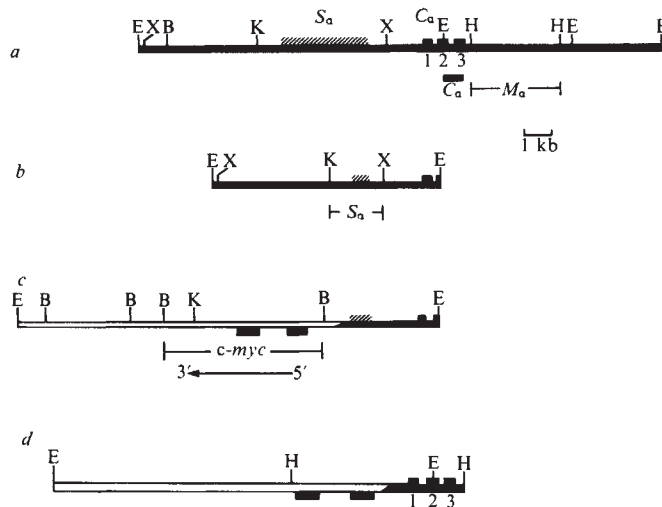


Fig. 2 Restriction maps of germ-line C_α and rearranged *c-myc*- C_α loci. Sites are abbreviated as follows: E, *Eco*RI; X, *Xba*I; B, *Bam*HI; K, *Kpn*I; H, *Hind*III. **a**, Restriction map of the germ-line C_α gene in the BALB/c mouse strain¹⁹. The regions coding for the C_{H1} , C_{H2} and C_{H3} domains of the α H-chain constant-region (C_α) are indicated by the numbered boxes 1, 2, 3 respectively. The area encompassing the switch region for the α H chain (S_α) is indicated by the cross-hatched box. The membrane exon, encoding sequences present on membrane-associated α H protein, is localized within the region indicated M_α . **b**, Restriction map of the λ CH4A 142.4 genomic clone²⁰ (supplied by F. Blattner). This clone of the germ-line C_α gene has a deletion of ~2 kb in the switch region, in the approximate position indicated. The switch region probe was prepared as described below. **c**, Restriction map of the genomic clone CH 315.1 of the *c-myc*- C_α locus isolated from the MOPC 315 variant line V-1. Partial *Eco*RI-restricted DNA was cloned into λ Charon 4A DNA²¹, packaged into phage particles²² and the resulting plaques screened²³. The arrow indicates the sense direction of transcription of *c-myc*. **d**, Restriction map of the *c-myc*- C_α locus in HOPC 1 as obtained by Southern blot analysis, performed as described in Fig. 3 legend.

Methods: A C_α cDNA clone was isolated as described previously²⁴ using G-C tailing of double-stranded cDNA, prepared from MOPC 315 RNA, into the *Pst*I site of pBR322. The cDNA insert is 650 bp long and includes the C_{H2} and C_{H3} sequences indicated by the solid line labelled C_α in **a**. S_α probe was derived from an *Xba*I-*Kpn*I restriction fragment from the germ-line clone λ CH4A 142.4. Purified DNA was digested with the indicated enzymes and the fragments separated by electrophoresis in low-melting agarose. The fragments were visualized by ethidium bromide staining and the band of interest was excised, the agarose melted at 70 °C, and the DNA extracted once with phenol, which had been equilibrated with 1 M Tris, pH 7.6. The DNA was removed from the supernatant by ethanol precipitation and collected by centrifugation. The membrane exon probe (M_α) was isolated from a clone of the productive α H-chain gene from the mouse myeloma M603. CH603 $\alpha 6$ (ref. 25; supplied by L. Hood). Following digestion of purified CH603 $\alpha 6$ DNA with *Hind*III, the M_α fragment was purified as above. The *c-myc* sequence probe (provided by R. Taub and P. Leder) was prepared by cloning the indicated 5.5-kb restriction fragment from the excluded C_α allele of the IgA-producing mouse myeloma S107 into pBR322 (ref. 26).



2b), which is a normal part of the J_H - C_{H1} intervening sequence of a complete α H-chain gene. This probe hybridizes to the 7.5- and 6.5-kb C_α RNAs (Fig. 1c; this gel was electrophoresed longer than that in **a**). Cytoplasmic RNA from these cells was next hybridized to the mouse *c-myc* probe (Fig. 2c). A band of hybridization is clearly visible at 7.5 kb, and faintly indicated at 6.5 kb (Fig. 1d, Cy). These species co-migrate with the C_α cytoplasmic 7.5- and 6.5-kb species detected in a duplicate blot (Fig. 1e, Cy). Thus these two large transcripts may contain sequences for both C_α and *c-myc*.

To begin to localize the sites of initiation and termination of transcription of the V-1 C_α RNAs, nuclear RNA was isolated and the size of the nuclear species determined in duplicate RNA blots (Fig. 1d, e). For C_α and *c-myc*, the largest species detected is 7.5 kb. Co-migration of these bands is observed. Cytoplasmic

and nuclear 7.5- and 6.5-kb species are similar in size, although minor differences in size would not be detected. Additional nuclear species migrating at 3.8 and 3.4 kb are seen for C_α and *c-myc* respectively.

The orientation of transcription is not determined by these experiments. Presumably a co-transcript could only be in the sense orientation for one of the two genes. To determine the direction of C_α RNA transcription, a C_α cDNA insert was cloned into phage M13. Single-stranded probes for C_α sense and anti-sense were isolated and labelled by a kinasing procedure we have developed (Fig. 1f). Hybridization with these probes demonstrates that all of the V-1 C_α RNA species are transcribed from the immunoglobulin-gene sense strand (Fig. 1f). Similar results are obtained with other procedures for labelling M13 probes (data not shown). The C_α RNAs in HOPC 1 are also

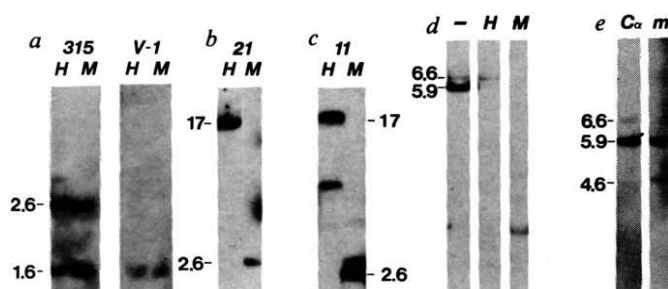


Fig. 3 Methylation of C_α alleles. **a**, Genomic DNA (10–15 μ g) from wild-type MOPC 315 or from the V-1 line was digested with either *HpaII* (H) or *MspI* (M). Following chloroform extraction, the DNA was electrophoresed on 1.4% agarose gels, transferred to cellulose nitrate paper²⁷ and the blot hybridized to the C_α cDNA clone as described in Fig. 1 legend for RNA blots. The 2.6-kb fragment from wild-type cell DNA is derived from the productive α allele²⁸ while restriction of the excluded allele in both lines gives rise to a 1.6-kb fragment. **b**, Genomic DNA was digested with *Bam*HI, extracted with chloroform and ethanol-precipitated. Aliquots (10 μ g) were digested further with *HpaII* or *MspI* and electrophoresed. Resulting blots were hybridized to the C_α cDNA clone. The C_α germ-line gene gives rise to a 17-kb *Bam*HI fragment. **c**, HOPC 1 genomic DNA was digested with *Hind*III, purified as above and 10- μ g aliquots digested with either *HpaII* or *MspI*. The blots were then hybridized to the C_α cDNA clone. **d**, Southern blot of HOPC 1 DNA digested with *Hind*III was probed with C_α cDNA. In this case, the gel was electrophoresed longer than in **c**. Subsequently, the blot was washed twice with 50% formamide, 1 mM EDTA, 1 $\times$ SSC for 30 min at 70 °C and then rehybridized with the *c-myc* probe (**m**).

transcribed from the C_α sense strand (M.D. and G.E.S., in preparation). These results suggest that transcription initiates at a promoter on the anti-sense strand of *c-myc*, proceeds through the switch region and terminates either within or close to the 3' end of the C_α gene.

To determine whether any of the transcripts from this locus could act as templates in polypeptide chain synthesis, their presence in polysomes was monitored (Fig. 4a). The small C_α species (transcripts between 800 and 2,400 bases) can be isolated predominantly from the large and small polysome regions, suggesting that they are in polysomes (Fig. 4a). To confirm this, polysome preparations were dissociated by EDTA treatment before sucrose gradient separation⁸ (Fig. 4b). Most of the C_α species shifted into the 30–60S region, demonstrating that these messages are present on polyribosome structures. The two large RNA species of 6.5 and 7.5 kb are found in the 80S and small polysome regions and show no significant shift in mobility on treatment with EDTA. This suggests that these messages are present primarily as ribonucleoprotein (mRNP) particles and are not active in protein synthesis⁹.

For *c-myc* RNA species, a band of hybridization at 2,400 bases is visible predominantly in the large polysome region (Fig. 4c). Species at 2,000 and 1,500 are detected in both the large and small polysome regions. Two species are present in both the small polysome and mRNP regions of the gradient: 3,000 and 2,500 bases. If the untranslated regions are not significantly larger in these mRNAs, they would seem to be translated less efficiently than the 2.4- and 2.0-kb *myc* RNAs. All the hybridization to these RNAs in the polysome regions disappears on EDTA treatment (data not shown), indicating that they are being translated. By contrast, hybridization is detected to a band at 7.5 kb and perhaps to one at 6.5 kb, which are present in the small polysome–80S region, and do not shift with EDTA treatment. Thus all the smaller C_α RNA species and many of the small *c-myc* species are present in polysomes, and are presumably templates for polypeptide chains.

Several pieces of evidence suggest that the 7.5-kb and possibly the 6.5-kb C_α RNA species in MOPC 315 V-1 cells are co-transcripts of C_α and *c-myc*. These large RNA species do not

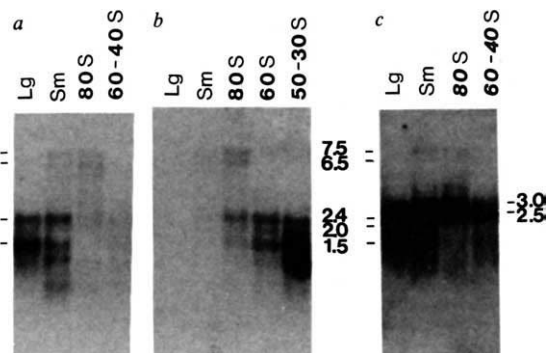


Fig. 4 Polysomal RNA analysis. Cultures of V-1 cells were chilled rapidly, washed twice with 20 mM Tris, pH 7.6, 120 mM KCl, 5 mM MgCl₂ and resuspended in 10 mM Tris, pH 7.6, 10 mM KCl, 1 mM MgCl₂ for 5 min on ice. Cells were lysed by the addition of Triton X-100 to 0.5%. After 5 min, the nuclei and mitochondria were removed successively by centrifugation at 4 °C for 10 min at 500g and at 12,000g respectively. The resulting supernatant was divided in half, and one aliquot was layered onto a 10–40% sucrose gradient containing 50 mM Tris, pH 7.6, 100 mM NaCl, 1 mM MgCl₂. The other half was treated with 40 mM EDTA for 10 min on ice before layering onto a similar sucrose gradient containing 25 mM EDTA. Following centrifugation at 26,000 r.p.m. for 3.5 h at 4 °C in an SW27 rotor, the gradients were monitored for absorbance at 257 nm and 0.5-ml fractions collected into 50 μ l of 5% SDS. The fractions were divided into large polysomes (Lg), small polysomes (Sm), 80S and 40–60S regions, pooled and the RNA extracted⁸. Poly(A)-containing RNA was then selected by oligo(dT)–cellulose chromatography and electrophoresed on 1% agarose–formaldehyde gels and hybridized as described in Fig. 1 legend. **a**, Untreated polysomal RNA hybridized to C_α cDNA. **b**, EDTA-treated polysomal RNA hybridized to C_α cDNA. **c**, Untreated polysomal RNA blot used in **a** was washed and hybridized to the mouse *c-myc* probe.

hybridize to DNA downstream from the C_H3 domain of the C_α region, while hybridization can be observed with probes including the switch region and the *c-myc* oncogene. Hybridization of the 6.5-kb RNA to the latter probe is always more tentative and probably indicates the presence of fewer *c-myc* sequences in this RNA. Similar *c-myc* and C_α -containing RNA species are present in fairly high levels in the nucleus of V-1 cells. Lastly, the C_α -hybridizing and *c-myc*-hybridizing large RNA species are present in mRNP particles of similar size, and do not seem to be active in protein synthesis. These results, while circumstantial, strongly suggest that an RNA product is transcribed across this locus.

As has been demonstrated for other myeloma lines^{5,9}, transcription of the translocated *c-myc* gene in V-1 and HOPC 1 cells also occurs from the *c-myc* sense strand (M.D., unpublished observations). Thus both strands of this locus are transcriptionally active. These events suggest the presence of an enhancer element. Such sequences have been found near the μ H-chain constant-region gene¹⁰.

The multiplicity of cytoplasmic C_α and *c-myc* RNA species produced from the translocated gene elements is unusual. Many of these RNA species are apparently active in protein synthesis. We have observed C_α -like polypeptides, immunoprecipitable with anti-IgA antiserum, in both V-1 and HOPC-1 cells (in preparation). These results raise important questions about the interdependence of translocation of *c-myc*, production of immunoglobulin-like RNA and protein structures, and the transformed state of myeloma and lymphoma cells.

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Lack of correlation between histone H4 acetylation and transcription during the *Physarum* cell cycle

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The interaction between nucleosomal proteins and DNA is expected to change during DNA replication as well as during transcription. A possible way of achieving the necessary structural changes is the modification of histones and high mobility group (HMG) proteins. The acetylation of core histones has been studied in various systems (for a review see ref. 1) and a correlation between histone acetylation and transcriptional activity of chromatin has frequently been proposed^{2–4}. In particular, Bradbury and co-workers^{5,6} have reported a cell cycle dependence of histone H4 acetylation in *Physarum polycephalum* which revealed two correlations: (1) tetraacetylated H4 (H4Ac₄) correlated with the rate of transcription and (2) H4 acetylation was inversely correlated with H1 phosphorylation in mitosis. We present evidence here that H4 acetylation does not fit these correlations. Our data clearly show that the acetate content of H4 is high during the S phase, but low during later stages of the cell cycle. H4Ac₄ remains at a nearly constant level during the whole cycle, with an elevation during the S phase. Furthermore, experiments with the deacetylase inhibitor sodium-*n*-butyrate do not support the proposed^{5,6} connection between diacetylated H4 (H4Ac₂) and DNA replication. Our data imply that a correlation of H4 acetylation and transcription is unlikely during the cell cycle of *Physarum*. The conclusions of Bradbury and co-workers are therefore invalid.

The myxomycete *Physarum polycephalum* is of special interest because its mitosis occurs with natural synchrony. It thus seems reasonable to exploit this characteristic to investigate correlations between various cell cycle-dependent events—in the case of this report, that between H4 acetylation and DNA replication

or transcription. Bradbury and co-workers^{5,6} reported that H4 acetylation fluctuates in a biphasic manner during the cell cycle, with maxima in the S phase and late G₂ period. They claimed that the percentage of H4Ac₄ also follows this biphasic rhythm. This was interpreted as a clear correlation with the rate of transcription, which also exhibits a biphasic cell cycle pattern⁷; moreover, the authors established a relationship between H4Ac₂ and DNA replication.

Because we had been unable to reconcile this with the cell cycle-dependent effects of the deacetylase inhibitor sodium-*n*-butyrate⁸, we reinvestigated H4 acetylation in *Physarum*. Figure 1 shows gel scans of the H4 region in 48-cm long polyacrylamide gels of histones from exponentially growing microplasmodia without (Fig. 1a) and with 1 mM butyrate in the culture medium (Fig. 1b). The H4 acetate content rises from 1.06 in control microplasmodia to 1.32 in microplasmodia grown in the presence of butyrate for 1 h (Fig. 1c). This hyperacetylation is mainly due to an increase of H4Ac₂ and a corresponding decrease of non-acetylated H4 (H4Ac₀) (Fig. 1d).

Figure 2 shows the acetate content (*N*) of H4 and the distribution of H4 subspecies during the synchronous mitotic cycle of *Physarum* macroplasmodia. The acetate content (Fig. 2, upper part) has a pronounced maximum in the S phase and then declines with progression of the cell cycle to a minimum in the G₂ period. It begins to rise again shortly before the onset of mitosis to reach its maximum in the subsequent S phase. This pattern clearly contradicts previously published data^{5,6}; we could not find a second maximum in the G₂ period.

Major changes occurred in the distribution of H4Ac₀–H4Ac₂. H4Ac₀ is at its lowest level in the S phase and rises at the end of S to reach a maximum before mitosis (Fig. 2, lower part). H4Ac₁ shows the smallest variation during the cell cycle in relation to the percentage of total H4 (~45%). It is high in telophase of mitosis and falls to a minimum at the S/G₂ border. H4Ac₂ exhibits the opposite pattern to H4Ac₀, with a maximum in the S phase and a minimum at the very beginning of mitosis. The fluctuation of H4Ac₂ is the most marked in relation to the percentage of total H4. H4Ac₃ is higher during the S phase, although the changes are relatively small. H4Ac₄ comprises only 3–4% of total H4, with a higher value in the S phase than the G₂ period. The previously reported biphasic^{5,6} pattern of H4Ac₄ was never detected.

Published data support the idea of increased accessibility of newly synthesized histones to acetylating enzymes⁹ compared with 'old' histones. This correlates well with our peak of acetate content in the S phase. Bradbury and co-workers^{5,6} found a relatively high acetate content of H4 during mitosis 2 (*N* = 1.1–1.2), whereas the value for mitosis 3 was considerably lower (*N* = 0.95), indicating that the experimental deviation is greater than the actual difference in acetate content which was considered to be highly significant; this was mainly due to the variation of H4Ac₂ and H4Ac₄, which were higher in mitosis 2 than in mitosis 3. The authors considered this to be due to changes in the composition of the culture medium. We checked this assumption by performing experiments in which the medium was replaced by fresh medium at different times, but we found that the acetylation pattern remained unchanged. We suppose these deviations to be artefacts due to the poor resolution of the 16-cm gel or the fact that nuclei and histones were isolated from frozen plasmodia. Furthermore, the authors claimed that the minimum acetate content occurred during prophase; this would be inversely correlated with H1 phosphorylation. We find this minimum in late G₂ together with a minimum of H4Ac₂–H4Ac₄; even before the onset of mitosis, the H4 acetate content has begun to rise to the S phase maximum. We always found a slightly higher acetate content in prophase (15 min before telophase) than in the very early stage of mitosis (preprophase, 25–30 min before telophase). As *Physarum* lacks a G₁ period, DNA synthesis starts immediately after mitosis, with a maximum rate of DNA synthesis occurring shortly after telophase (see ref. 10 for a review). It seems logical that structural changes in chromatin necessary for DNA replication must occur before or

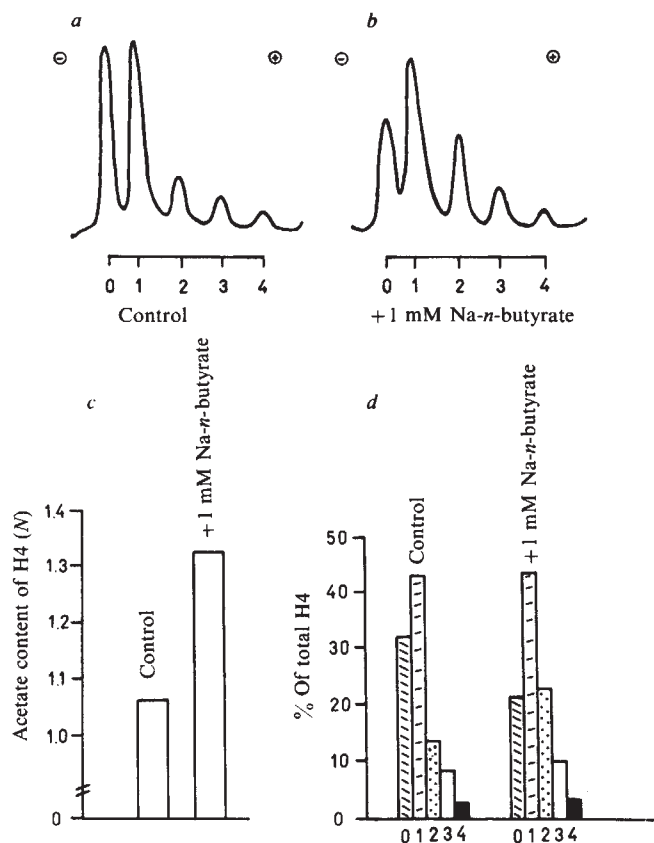


Fig. 1 Polyacrylamide gel electrophoresis¹⁷ of H4 from exponentially growing microplasmodia of *Physarum polycephalum*. *a*, Gel scan of H4 region of control microplasmodia: 0–4 indicates the number of acetates per H4 molecule. *b*, Microplasmodia grown in the presence of 1 mM butyrate for 1 h. *c*, *N* of control- and butyrate-treated microplasmodia (as in *a*, *b*). *d*, Percentage of H4Ac₀–H4Ac₄ in control and butyrate-treated microplasmodia.

Methods: Microplasmodia (strain M_{3b}, Wis1 isolate) were maintained in submersed culture in semi-defined medium as described elsewhere¹⁸. Histones were extracted from isolated nuclei¹⁹ according to a published procedure²⁰; nuclear isolation was performed immediately after collection of plasmodia, as isolation of nuclei from frozen plasmodia leads to artefacts in the histone pattern. In particular, histones from butyrate-treated cultures can only be isolated immediately from unfrozen plasmodia. Butyrate (100 mM) was routinely included in all buffers for isolation of nuclei and histones to prevent deacetylation during the processing of the samples; this leads to a slightly elevated acetate content (*N*) compared with histone samples where butyrate was omitted during isolation. Phenylmethylsulphonyl fluoride and mercaptoethanol were included in buffers to prevent proteolysis and oxidation. Histones were subjected to polyacrylamide gel electrophoreses in acid urea²¹ as well as in acid urea Triton X-100 gels¹⁷. Every histone sample was run at least twice on both gel systems. Gels were 48 cm long so as to achieve a satisfactory resolution of H4. Results were the same regardless of the thickness of the gel (0.75–1.5 mm). Gels were stained with Coomassie blue, destained by diffusion, and dried. The clearly separated H4 bands, fitting a gaussian distribution, could be easily quantified in a Beckman-DU-8 spectrophotometer with a gel scanning appliance. Bradbury and co-workers⁵ used 16-cm long gels and analysed their H4 scans with a computer to correct for the gaussian distribution of peaks. Apart from the poor resolution of the H4 region in 16-cm gels, the authors purified their histones by gel chromatography to eliminate a minor band interfering with H4Ac₄; this minor component is often co-extracted with the bulk histones, but is separated clearly from the whole H4 region in 48-cm gels. We could therefore omit this additional chromatography step, which is a possible cause of artefacts. The acetate content (*N*) was calculated by the formula

$$N = \frac{\sum_{n=1}^4 nP_n}{\sum_{n=0}^4 P_n}$$

where P_n is the percentage of total H4 occurring as the subspecies with *n* acetates.

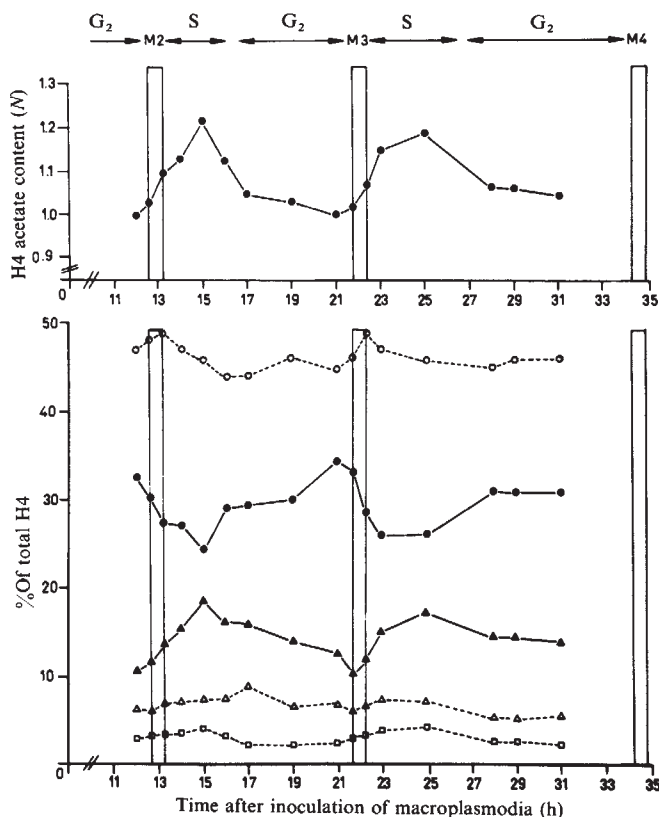


Fig. 2 Acetate content (*N*) and percentage of acetylated subspecies of H4 histone during the naturally synchronous cell cycle of macroplasmodia of *Physarum polycephalum*. Lower part: ●, percentage of H4 in nonacetylated form; ○, percentage of H4 in monoacetylated form; ▲, percentage of H4 in diacetylated form; △, percentage of H4 in triacetylated form; □, percentage of H4 in tetraacetylated form. M2, M3, M4: mitoses 2, 3 and 4, respectively; arrows (S, G₂) indicate the approximate duration of the S phase and G₂ period, respectively. Columns indicate the duration of mitosis from preprophase to telophase.

during the early stages of mitosis. Enzymes involved in DNA synthesis are also induced before mitosis in *Physarum*^{11,12}. Nevertheless, it remains to be investigated whether such small changes in acetate content (from 1.0 in preprophase to 1.1 in telophase) have any significant function in the chromatin alterations necessary for DNA replication.

Our reinvestigation of the cell cycle pattern of H4 acetylation revealed an interesting correlation between H4 acetate content and the effect of butyrate⁸. Butyrate delays mitosis and leads to a prolongation of the cell cycle when it is applied in those stages of the cycle where H4 acetate content is high (S phase, early G₂ period); whether this is a mere coincidence or a causal connection remains to be clarified.

There are many reports which correlate histone acetylation with transcriptional activity of chromatin (for reviews see refs 1, 13). Enhanced template activity for RNA synthesis was shown for hyperacetylated chromatin. Treatment of exponentially growing *Physarum* microplasmodia in asynchronous culture with 1 mM butyrate leads to hyperacetylation (Fig. 1*b–d*). This hyperacetylation was found to be reversible, showing a maximum (*N* = 1.5) after ~5 h and a subsequent decline to the initial control value (*N* = 1.03) 24 h after the start of treatment (Fig. 3, upper part). We recently showed⁸ that butyrate causes a reversible inhibition of the synthesis of DNA, RNA and protein which follows the same pattern as H4 hyperacetylation. It is interesting that the increase in H4 acetate content in the S phase of the cell cycle as well as during butyrate-induced hyperacetylation is mainly due to an increase in H4Ac₂ and a corresponding decrease in H4Ac₀ (Fig. 3, lower part). H4Ac₃ and H4Ac₄ make minor contributions to the hyperacetylated state. After 24 h all H4 subspecies return to nearly the initial control values.

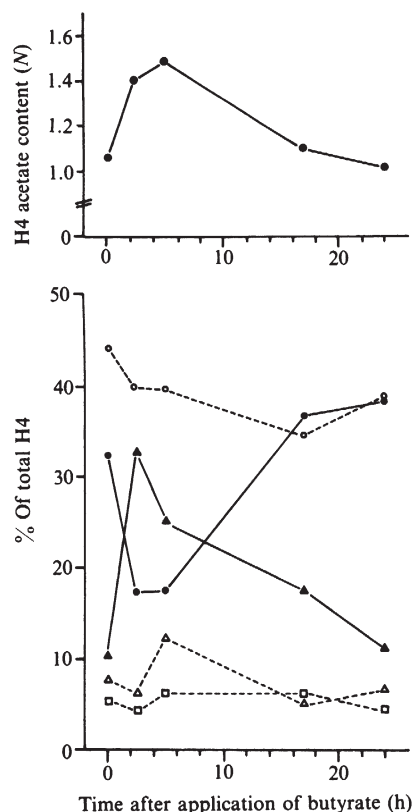


Fig. 3 Acetate content (N) and percentage of acetylated sub-species of H4 histone of *Physarum* microplasmodia grown in the presence of 1 mM sodium-*n*-butyrate. Lower part: ●, percentage of H4 in nonacetylated form; ○, percentage of H4 in monoacetylated form; ▲, percentage of H4 in diacetylated form; △, percentage of H4 in triacetylated form; □, percentage of H4 in tetraacetylated form.

Bradbury and co-workers^{5,6,14} have postulated a tight correlation between H4 acetylation and the rate of RNA synthesis in *Physarum*, as both were thought to have a biphasic cell cycle pattern. Our data contradict this, as we find only one peak of H4 acetylation in the S phase. This acetylation pattern does not rule out a possible relationship between acetylation and transcription, as RNA synthesis does not necessarily follow this extreme biphasic oscillation; RNA synthesis measured in isolated nuclei of *Physarum*¹⁵ revealed a maximum in the S phase, which was due to a peak in RNA polymerase B activity, whereas the nucleolar polymerase A remained constant throughout the cycle. Our results with butyrate-treated hyperacetylated microplasmodia, which are inhibited in RNA synthesis⁸, do not support a connection between H4 acetylation and transcription *in vivo*. Furthermore, the fact that butyrate leads to a dramatic increase of H4Ac₂ in conditions of nearly complete inhibition of DNA and protein (histone) synthesis⁸ does not favour a connection between diacetylation of H4 and DNA replication^{5,6,14}.

Thus, from our data we cannot conclude that the acetylation of H4 is as important for cellular events as has been proposed^{5,6,14}; in particular, our results with butyrate cast doubt on the role of acetylation in transcription. Recent data support the concept that other factors are of more importance for the transcriptional activity of chromatin; for example the coordinate removal of H2A and H2B in connection with HMG proteins¹⁶.

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Viability of λ phages carrying a perfect palindrome in the absence of recombination nucleases

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In *Escherichia coli* *in vitro* constructions of perfect palindromes larger than 30 base pairs (bp) long have in general been unstable^{1–8}. A perfect palindrome has the unique possibility of forming a cruciform structure, and it is this feature which probably results in its instability. Negative supercoiling favours the formation of the cruciform conformation, which in turn causes the molecule to relax^{1,2}. This relaxation may render replicons containing large perfect palindromes inviable⁴. An alternative hypothesis for inviability has been that the cruciform interferes with replication by favouring strand switching by polymerase I³. Here we show that the simultaneous absence of two recombination nucleases, the *recBC* product, exonuclease V, and the *sbcB* product, exonuclease I, confers viability on a derivative of phage λ carrying a perfect palindrome of inverted repeat length 1,600 bases. This observation suggests a third hypothesis—that nucleolytic cleavage of the cruciform is responsible for the inviability of the phage. Such an activity has been shown *in vitro* for T4 exonuclease VII¹⁰.

A perfect palindrome of the non-essential 1,600-bp λ *XbaI*–*EcoRI* fragment was constructed between *EcoRI* sites 1 and 2 of phage λ thus replacing the non-essential *EcoRI* B fragment. This was done by *XbaI* restriction of λ DNA with a normal *EcoRI* B fragment and with an inverted *EcoRI* B fragment (designated B') followed by ligation to form a perfect palindrome (see Fig. 1). *XbaI* was used in this experiment because it recognizes a single target site in phage λ that lies within the *EcoRI* B fragment. In this manner one Red⁺ and one Red[–] palindrome-containing phage were constructed. Control Red⁺ and Red[–] phages carrying a normal (non-palindromic) *EcoRI* B fragment were also made, using the same method, from parental phage both carrying the normal *EcoRI* B fragment. Following *in vitro* packaging, the desired particles were selected on a *su*[–] indicator strain by the absence of *susA* or *susP* markers in the left and right arms of the parental phage (see Fig. 1).

The ability to select for the phages containing the palindromes allowed their plating behaviour to be studied in different strains. Table 1 shows the results of a survey of strains carrying mutations in genes affecting recombination. Clearly, the palindrome-containing phages can plate out for plaques with an efficiency approaching that of the control phages, only on strains carrying

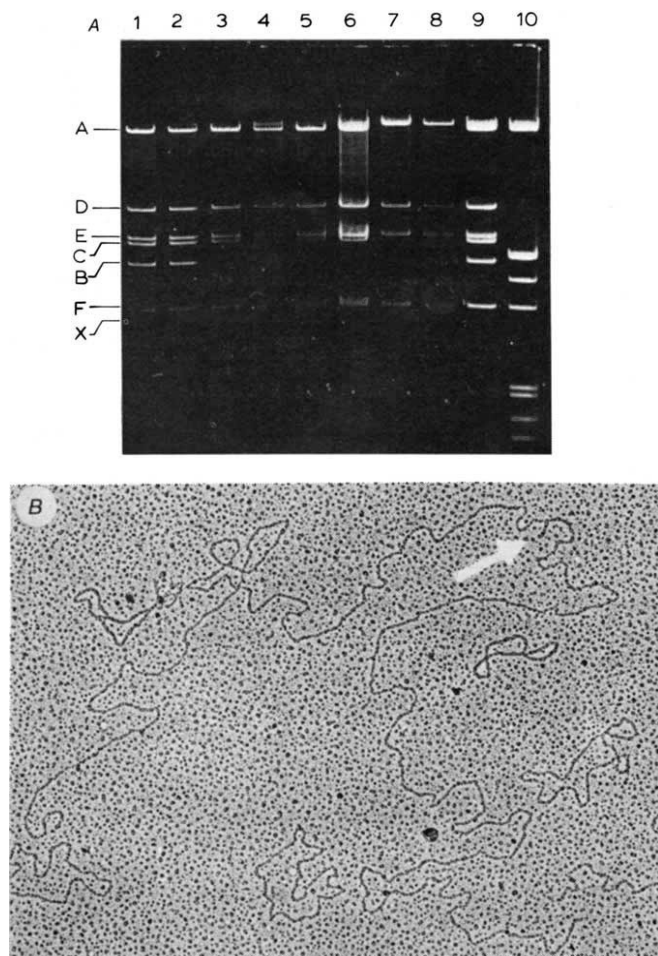


Fig. 1 A, B, Construction of the Red⁺ palindrome-containing phage and its control. C, *EcoRI*-*XbaI* restriction map of phage λ . The *EcoRI* sites are designated by short vertical bars and divide λ into fragments A-F, the sizes of which are given in kb. The single *XbaI* site divides the 4.9-kilobase (kb) *EcoRI* B fragment into a 3.3-kb and a 1.6-kb fragment.

Methods: The *red3* derivatives were constructed in an identical manner using DRL34 (*susA32*, *red3*, *sus gam 210*, *cl857*) instead of DRL32 (*susA32*, *sus gam 210*, *cl857*). Phage strains MMS997 (ΔC , *sus gam 210*, *cl168*, *susP80*) and MMS999 (B' , ΔC , *sus gam 210*, *cl168*, *susP80*) were grown as plate stocks on *E. coli* strain GM119 (*dam*), and phage strains DRL32 and DRL34 were grown by induction of lysogens of GM119 to prevent Dam methylation of the *XbaI* site. The phage particles were concentrated by high-speed centrifugation and were then purified twice by CsCl equilibrium density centrifugation. Phage DNA was isolated by treatment with SDS and phenol and dialysed extensively against TE buffer (10 mM Tris, 1 mM EDTA, pH 7.5). Restriction of 2 μ g of each DNA with 5 units of *XbaI* (BRL) was done in 150 mM NaCl, 6 mM MgCl₂, 6 mM Tris pH 7.9 for 1 h at 37 °C. The enzyme was heat-denatured and the buffer changed by dialysis against ligation buffer (150 mM Tris, 20 mM MgCl₂, 6 mM EDTA, 50 mM dichlorodiphenyltrichloroethane). Appropriate DNAs were mixed, 1 mM ATP and 250 μ g ml⁻¹ bovine serum albumin were added, and the DNA was ligated with 50 units of T4 ligase (NE Biolabs) at 14 °C for 20 h. The ligated DNA was dialysed against TE buffer before *in vitro* packaging using the method of Kobayashi and Ikeda¹⁶ with the modification that putrescine was added to increase the efficiency of packaging of shorter DNAs. Packaging extracts were prepared from *E. coli* lysogens NS428 and NS433 (ref. 17). Selection against phage particles carrying the *susA32* or *susP80* mutations was accomplished by plating on *su*⁻ indicator strains. To confirm that the desired palindromic DNA had been formed, samples were digested with *XhoI* (which cuts once in the right arm of λ), denatured and spread after short reannealing times to detect the presence of any snapbacks. As expected, snapbacks were seen, which measured 1.60 ± 0.14 kb (Red⁺ phage) and 1.60 ± 0.10 kb (Red⁻ phage), which corresponds to the expected length of the palindrome selected for in the experiment.

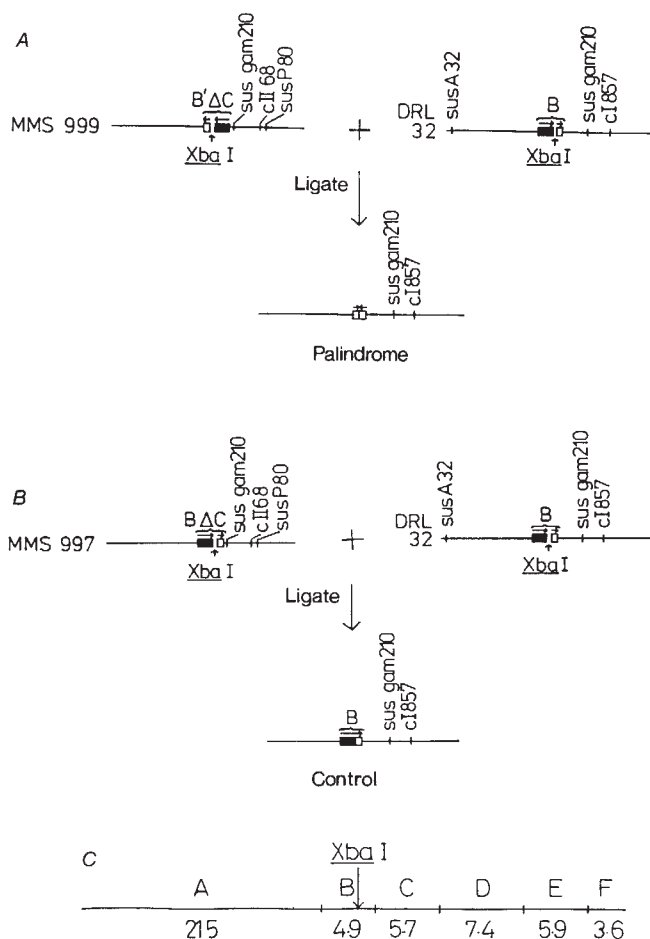


Fig. 2 A, *EcoRI* restriction digests of the DNA from phages derived from particles containing perfect palindromes. Lanes 1-4: the digests shown are of DNA obtained from plate lysates grown on *recBC sbcB* bacteria, each of which originated from 20 pooled plaques obtained from a plate seeded with *recBC sbcB* bacteria and the *in vitro*-packaged phage. Lane 1, Red⁺ control phage; 2, Red⁻ control phage; 3, Red⁺ palindrome-containing phage; 4, Red⁻ palindrome-containing phage. Note the absence of bands B and X from tracks 3 and 4. Lanes 5-8: the digests shown are of DNA obtained from plate stocks of phages derived from palindrome-containing particles that were originally plated on *recBC sbcB* bacteria but which had acquired the ability to plate with equal efficiency on Rec⁺ bacteria. Lanes 5 and 6, Red⁺ phage; lanes 7 and 8, Red⁻ phage. In all four tracks bands B and X are absent; tracks 7 and 8 also have no band C, while track 5 shows a new band of 1.3 kb. These patterns are all consistent with deletions having occurred in the region of the palindrome. Lane 9, *EcoRI* restriction digest of λ cl857; 10, *EcoRI* + *HindIII* restriction digest of λ cl857. Bands A-F are labelled according to the standard nomenclature of λ *EcoRI* fragments (see Fig. 1C) and X designates the expected migration distance of the undelimited perfect palindrome. B, Electron micrograph of a 310-bp snapback seen in the DNA of the Red⁺ palindrome-containing phage after passage through the *recBC sbcB* host. Similar structures were seen in the DNA of the Red⁻ palindrome-containing phage. DS standard is pAT153 (3.657 kb), SS standard is M13 (6.407 kb).

the *recBC* and *sbcB* mutations. In *recBC sbcB* strains, the RecF pathway of recombination is activated¹¹. However, it is unlikely that this pathway is responsible for the plating of the palindrome-containing phages, as the introduction of the *recF143* mutation does not significantly reverse the effect of the *recBC* and *sbcB* mutations. When compared with its behaviour on the *recBC SbcB* strain, a fivefold greater decrease in plating efficiency was observed for the Red⁻ palindrome-containing phage relative to its control in the *recBC sbcB recF* strain DL148. However, this was not observed with the *recBC sbcB recF* strain JC11850 or

Table 1 Plating behaviour of palindrome-containing phages on strains carrying mutations in genes involved in genetic recombination

Strain	Recombination genotype	Control phages (ml ⁻¹)		Palindrome-containing phages (ml ⁻¹)	
		Red ⁺	Red ⁻	Red ⁺	Red ⁻
JC9937	<i>rec</i> ⁺	2.6 × 10 ⁵	2.9 × 10 ⁵	—*	—
DL184	<i>recB21 recC22</i>	1.9 × 10 ⁵	2.4 × 10 ⁵	—	—
JC11477	<i>sbcB15</i>	2.9 × 10 ⁵	3.4 × 10 ⁵	—	—
JC9387	<i>recB21 recC22 sbcB15</i>	2.0 × 10 ⁵	2.4 × 10 ⁵	1.7 × 10 ⁵	1.4 × 10 ⁵
DL148	<i>recB21 recC22 sbcB15 recF143</i>	1.0 × 10 ⁵	1.6 × 10 ⁵	6.2 × 10 ⁴	2.0 × 10 ⁴
JC11850	<i>recB21 recC22 sbcB15 recF143</i>	2.9 × 10 ⁵	3.2 × 10 ⁵	1.8 × 10 ⁵	1.9 × 10 ⁵
JC9388	<i>recB21 recC22 sbcA23</i>	2.5 × 10 ⁵	2.8 × 10 ⁵	—	—

Plating cultures of the strains tested were grown to early stationary phase in tryptone broth supplemented with 0.2% maltose, 5 mM MgSO₄ and 20 µg ml⁻¹ vitamin B₁. They were then diluted twofold in buffer to give a final concentration of 5 mM Tris, 7.5 mM MgSO₄. All platings were done on trypticase plates at 37 °C. All strains are *su*⁻ and all except JC11850 are derivatives of AB1157 (ref. 14). The JC strains were obtained from A. J. Clark, and the DL strains are *Su*⁻ mutants of strains obtained from A. J. Clark. DL148 and DL184 derive from JC8111 and JC5519 respectively, and were constructed as described previously¹⁵.

* <10³ ml⁻¹.

Table 2 One-step experiments

		<i>rec</i> ⁺ / <i>recBC sbcB</i> plating ratios of the progeny from one-step growth experiments in:	
		<i>recBC sbcB</i>	<i>rec</i> ⁺
Control phages	Red ⁺	1.2	0.9
	Red ⁻	1.2	1.0
Palindrome-containing phages	Red ⁺	<0.03	—
	Red ⁻	<0.02	—

25 µl of *in vitro*-packaged phage and 25 µl of bacteria at 1.5 × 10⁸ ml⁻¹ were mixed and gently agitated at 34 °C for 20 min. Buffer (5 ml) was added to each mixture, which was then filtered through a 0.45-µm Millipore filter to remove unadsorbed phage. Bacteria held on the filter were washed into 5 ml of λ tryptone broth; 2 ml of the suspension were immediately treated with chloroform, and 0.1 ml was plated on the *recBC sbcB* indicator to test for unadsorbed phage. 3 ml of the suspension were aerated at 37 °C for 60 min then treated with chloroform to release the progeny phage and prevent readsorption to bacteria. The *recBC sbcB* and *rec*⁺ strains used were JC9387 and JC9937, respectively.

with Red⁺ phage on either strain and is probably due to the poor growth of Red⁻ λ plaques on DL148. Table 1 also shows that the presence of the Red pathway of recombination or the activation of the RecE pathway of recombination by the *sbcA* mutation is not sufficient for plating of the phages containing the palindrome.

The progeny phage obtained from six of the plaques formed by the palindrome-containing particles on the *recBC sbcB* host were tested for their ability to plate on Rec⁺ and *recBC sbcB* bacteria. Most of the progeny were still unable to plate on the Rec⁺ strain, but between 0.08% and 20% were able to do so. When 20 plaques were pooled and plate stocks grown on the *recBC sbcB* host, 16% of the Red⁺ phage and 57% of the Red⁻ phage were now able to plate on the Rec⁺ host. These observations suggested that despite the ability of the palindrome-containing phages to plate on *recBC sbcB* bacteria, their palindromes were unstable. This was confirmed by physical analysis of the DNA obtained from this same population of particles. The restriction fragment expected for the perfect palindrome was absent from both the Red⁺ and Red⁻ DNAs (see Fig. 2A). The fact that there was no physical evidence of the original palindrome, despite the large proportions of phages still unable to plate on a Rec⁺ host, can be understood if rearrangements had occurred in all progeny phage and if many of the rearrange-

ments resulted in structures which retained elements of palindromic symmetry. To test this hypothesis, the DNA from these same populations of phage particles was denatured and spread after short reannealing times and was observed under the electron microscope for the presence of snapbacks. Many snapbacks were observed with an average length of 300 ± 110 bp (Red⁺ phage) and 340 ± 90 bp (Red⁻ phage). Loop-stem structures were not seen, confirming that these new, shorter palindromes had little or no DNA separating the inverted repeats (see Fig. 2B). Deletions which are nearly symmetrical about the centres of palindromes have been demonstrated previously^{5,6}.

Restriction analysis was also carried out on DNA from four phages that had acquired the ability to plate on the Rec⁺ host. Here, as expected, deletions were observed in the region of the palindrome (see Fig. 2A).

To verify that the ability of the palindrome-containing phages to plate on a *recBC sbcB* host was due to increased survival of phages with parental plating properties, one-step growth experiments were done in *recBC sbcB* and Rec⁺ strains. Table 2 shows that growth of the palindrome-containing phages was not detected in the Rec⁺ host and that >97% of the progeny obtained from the *recBC sbcB* host retained the plating properties of phages containing perfect palindromes.

Following infection, λ DNA undergoes conversion from a linear to a closed circular supercoiled form in which we expect the palindrome to adopt the cruciform conformation. We propose that it is this structure which causes inviability in the presence of either exonuclease V or exonuclease I. This could be due to a direct action of these nucleases on the palindromic DNA or to an indirect effect of these enzymes, for example on DNA replication or on the level of supercoiling in the cell. Consistent with the direct action hypothesis is the fact that the centre of a cruciform structure is physically identical to the Holliday junction, a central intermediate in genetic recombination. It has been hypothesized that exonuclease V might cut diagonally across a Holliday junction to generate recombinant DNAs¹². If such a cut were made across the base of a cruciform in a closed circular molecule, the molecule would be linearized. It has also been hypothesized that exonuclease I acts on a recombination intermediate¹³, albeit an unspecified one. The observations reported here suggest that both exonuclease V and exonuclease I may be active in the resolution of Holliday junctions.

Collins *et al.*⁶ have recently shown that the precise excision from a plasmid of a nearly perfect palindrome bounded by short direct repeats is reduced in a *recBC sbcB* strain. Their observation may be consistent with the above hypothesis.

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Orientation of the primary donor in single crystals of *Rhodopseudomonas viridis* reaction centres

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Magnetic resonance studies have greatly helped the study of the primary photochemistry of bacterial photosynthesis. The full power of magnetic resonance techniques for structure determination of paramagnetic species can only be realized, however, when measurements are made on fully oriented samples such as single crystals. Thus the recent crystallization of the reaction-centre protein from *Rhodopseudomonas viridis*¹ has enhanced the amount of structural information about the reaction centre that can be obtained using these methods. It is well known that when the primary quinone acceptor of the reaction centre is prereduced, the primary photochemical charge separation lasts only about 20 ns before back electron transfer leads to the lowest excited triplet state of the primary donor². We now report the electron paramagnetic resonance (EPR) spectra of this photochemically derived triplet state of the primary donor of *R. viridis* in a single reaction-centre crystal as a function of crystal orientation in the magnetic field. Analysis of these spectra allows us to assign the orientation of the triplet-state axis system of the primary donor relative to the crystal axis system.

Reaction centres of *R. viridis* were prepared using the method described by den Blanken *et al.*³. The reaction centres had an optical absorbance ratio $A_{280}/A_{830} = 2.6$. SDS-polyacrylamide gel electrophoresis showed that the reaction centres consist of four types of protein subunit^{4,5}. The photochemical integrity of the reaction centres was checked by EPR following Thornber *et al.*⁶ and den Blanken *et al.*³ and shows that the first quinone acceptor, Q, in these particles can be photoreduced at low temperatures when sodium ascorbate is present to reduce the oxidized cytochromes.

Crystallization of the reaction-centre protein was accomplished using a slight modification of the Michel method¹. Briefly, a 1-ml reaction-centre solution having $A_{830} = 2$ and

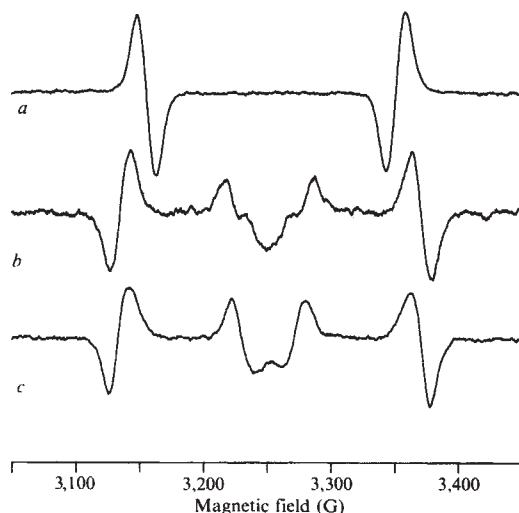


Fig. 1 First-derivative triplet EPR spectra for three different orthogonal crystal axes along the EPR magnetic field H_0 . *a*, H_0 parallel to the crystal *c*-axis; *b*, H_0 parallel to the crystal *a*-axis; *c*, H_0 parallel to the crystal *b*-axis. Instrument settings: microwave power 1 mW, modulation amplitude 16 G, temperature 5 K.

containing 50 mM phosphate buffer, pH 6, 3% (2*R*;3*R*,*S*)-1,2,3-heptanetriol, 0.1% lauryl dimethylamine oxide (LDAO) and 1.5 M $(\text{NH}_4)_2\text{SO}_4$ was equilibrated with about 4 ml of 2.5 M $(\text{NH}_4)_2\text{SO}_4$ at 21 °C. The dark brown crystals grew to a typical dimension of $0.7 \times 0.15 \times 0.15$ mm in 10 days. Examination of the crystals under a microscope revealed smooth surfaces and very sharp edges. On dissolving several crystals in 0.1% LDAO, 10 mM Tris, pH 8, the solution possessed an absorption spectrum identical to the original solution except that $A_{280}/A_{830} = 2.1$.

For the EPR measurements one crystal was carefully transferred to a solution containing 1.5 M sucrose, 2.5 M $(\text{NH}_4)_2\text{SO}_4$, 100 mM sodium ascorbate, 50 mM phosphate buffer, pH 6, and incubated for about 10 min. Together with a small drop of that solution the crystal was mounted on a goniometer and immersed in liquid nitrogen. The solution formed an optically clear glass. No visible damage to the crystal due to freezing or thawing was found using the above solution even after five freeze-thaw cycles. Reduction of Q was accomplished by photoreduction at 5 K. EPR triplet spectra of P_{990} were recorded using 1-kHz modulation of the xenon light source and lock-in detection². The spectra were averaged over four field sweeps. The phase of the lock-in amplifier was set to optimize the H_x -transition in the powder spectrum of the P_{990} triplet in a reduced reaction-centre solution of *R. viridis*.

Figure 1*a–c* shows triplet EPR spectra of the reaction-centre primary donor for orientations of the magnetic field along each of three crystal axes *a*, *b*, *c*. If no sodium ascorbate was added and the crystal was frozen in the dark, P_{990}^+ was generated on illumination at 5 K. Figure 2*a, b* shows the changes in the observed field at which resonance occurs for each line as a function of rotation about two of the crystal axes.

The most interesting feature of the observed spectra is the simple spectrum that results when the long *c*-axis of the crystal is parallel to the magnetic field direction (Fig. 1*a*). This shows that all the reaction-centre primary donors in the crystal are magnetically equivalent in this direction and suggests that the crystals contain one reaction centre per asymmetric unit (compare refs 1 and 7). Rotation of the crystal in any direction orthogonal to this direction results in, at most, an eight-line spectrum indicative of only four magnetically non-equivalent sites in the crystal. This agrees with the tetragonal $P_{4,2,2}$ symmetry of the crystals which are expected to have four magnetically non-equivalent sites, since sites related by a 2-fold axis (180° rotation) are magnetically equivalent.

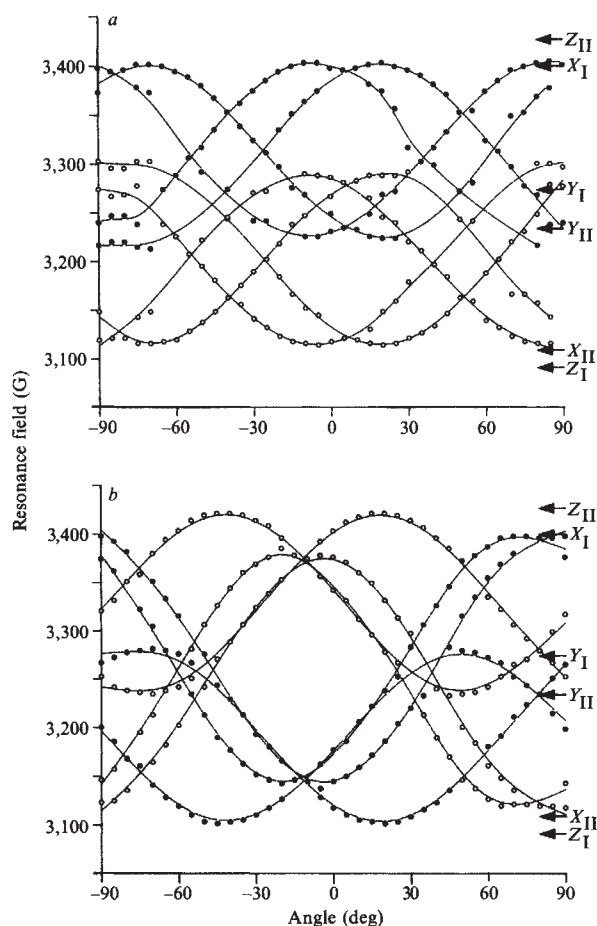


Fig. 2 The eight triplet EPR resonance positions as a function of magnetic field H_0 for rotation about the crystal c -axis with H_0 perpendicular to the c -axis (a) and for rotation about the a -axis with H_0 perpendicular to the a -axis (b). $\circ$, Emissive resonance lines; $\bullet$, absorptive resonance lines. $X_{I,II}$, $Y_{I,II}$ and $Z_{I,II}$ are the resonance positions if the magnetic field H_0 were along the triplet x -, y - and z -axes, respectively, with I denoting absorption and II denoting emission.

The changes in the electron spin polarization pattern exhibited by the spectra on rotation of the crystal are consistent with population of the triplet via the ST_0 radical pair mechanism². Thus, the observed triplet is a result of native photochemical charge separation in the reaction centre. The crystalline nature of the sample results in approximately a 1,500-fold gain in triplet EPR signal intensity compared with that observed for randomly oriented reaction centres. The angular dependences of the spectra shown in Fig. 2 can be analysed by known rigorous techniques and the spectra simulated to yield the geometry of the non-equivalent magnetic sites relative to the crystal axes (ref. 8 and Fig. 3A,B).

Each pair of non-equivalent sites is oriented 90° to the other along the 4-fold screw axis. This accounts for the simplicity observed in the spectrum in Fig. 1a. The angles between the crystal axes a , b , c and the triplet x -, y -, z -axes of one site are x , $a = 11^\circ$; x , $b = 83^\circ$; x , $c = 81^\circ$; y , $a = 100^\circ$; y , $b = 31^\circ$; y , $c = 61^\circ$; z , $a = 95^\circ$; z , $b = 121^\circ$; z , $c = 31^\circ$, all $\pm 1^\circ$. The orientations of the remaining sites relative to the crystal axis system are fixed by the $P_{4,2,2}$ space group.

Recently, the dependence of the optical absorption spectrum of the crystal on the polarization of the measuring light was determined⁷. These workers suggested that the Q_y optical transition of the primary donor chromophore is perpendicular to the crystal long c -axis. Our triplet state data determine the relative orientation of the chromophores to $\pm 1^\circ$ and yield a vastly more detailed picture.

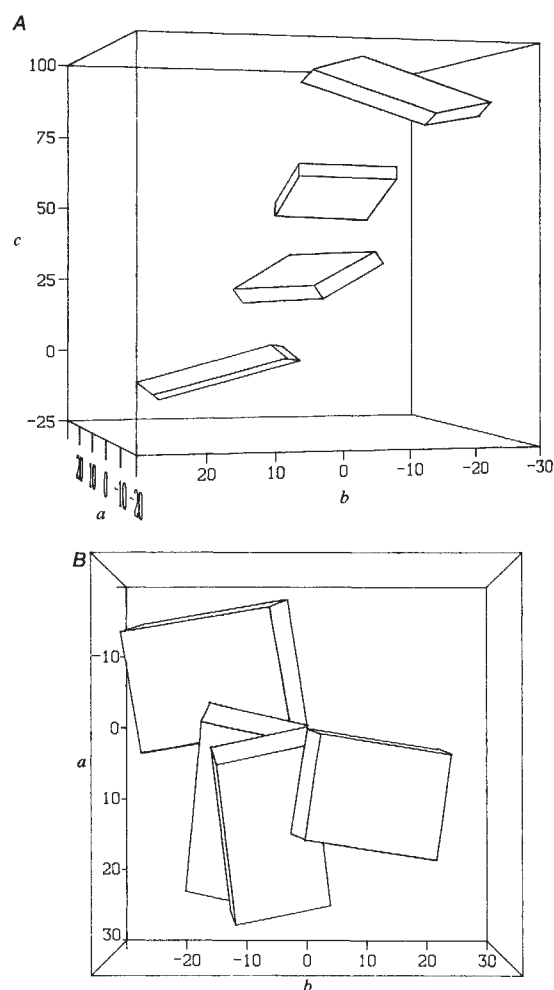


Fig. 3 A, Diagram depicting in the crystal axis system (a , b , c) the relative geometry for the four distinguishable magnetic sites. The rectangular boxes represent the triplet axis system of each distinguishable magnetic site where the x -axis is the medium-length dimension, the y -axis the longest dimension and the z -axis the shortest dimension of the boxes. B, View looking down the crystal c -axis. Distances between the four sites are not intended to be to scale.

Thus we have used the photoexcited triplet state of the primary donor of *R. viridis* to determine precisely the relative orientation of the magnetically non-equivalent donor sites and corresponding reaction-centre asymmetric unit relative to the crystal axes. Our results show conclusively that there are only four such magnetically non-equivalent sites and that, remarkably, all sites become magnetically equivalent when the magnetic field is oriented parallel to the crystal long c -axis.

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People and particles

Philip Davenport

The Infancy of Atomic Physics: Hercules in his Cradle.

By Alex Keller.

Clarendon Press/Oxford University Press: 1982. Pp.230. £12.50, \$18.95.

AWESOME and austere were the sepia portraits of famous men of science that looked down from our classroom walls or gazed somewhat reproachfully from our textbooks. Such demigods, we thought, were never a prey to human foibles and fallibilities; their hearts never ruled their heads nor their passions coloured their judgement. Alex Keller shows us just how mistaken we were.

The great merit of his history of atomic physics is the clarity and insight with which the leading figures are portrayed. This extends to their family backgrounds, their personalities, their domestic affairs and in some cases their courtships which are so sensitively described that the reader soon feels an almost uncanny empathy with them. This is moreover achieved without a hint of prurient irrelevance; Keller is far removed from the genre of gossip columnists.

As regards the subtitle, Hercules symbolizes the tremendous potential for good and evil of atomic physics. His infancy sees the gradual and painstaking evolution of the atomic model which today we take for granted. Ultimately (to adapt Milton) this noble and puissant science, mewing its mighty youth, presents mankind with the invidious options of nuclear energy and nuclear weapons.

The story begins in the 1880s with the speculations and experiments of William Crookes. Versatile, imaginative and enterprising, he was by far the most flamboyant figure in British science. He is the first of a formidable galaxy of principal investigators to whom we are introduced, men of genius whose names are household words and, in many cases, SI units. We meet them in their home environment, become acquainted with their circumstances, abilities and ambitions, and accompany them on their journeys of exploration and elucidation. Progress demanded the gradual assimilation of the fruits of many diverse lines of patient research and fortuitous discovery, whose interrelation was initially unsuspected. Such phenomena as cathode rays, X-rays, radioactivity, isotopes, nuclear transmutation and ultimately nuclear fission were one by one embodied into the grand design until no loose ends remained.

Many separate accounts have been

written of the scientific development of these subjects, mainly in the context of biographies and memoirs. There are also surveys of contemporary scientific advances during the period, but they tend to be impersonal and idealized in approach. Keller has deliberately set out to achieve what few historians have attempted, a synthesis of both the subjective and objective approaches, and he has succeeded in producing an honest account of man's erratic progress towards the concept of the nuclear atom as it happened. His book well illustrates the validity of Watson's remark in *The Double*



J.J. Thomson in his laboratory.

Helix, that "science seldom proceeds in the straightforward logical manner imagined by outsiders" but rather by stages which are often "very human events in which personalities and cultural traditions play major roles".

Keller contrasts the differing national attitudes to physical science at the turn of the century, when in his words, "the language of racial competition and the imagery of war and soldiering came easily to the tongue". The British stressed the value of models and analogies, the French looked for their theories to lead to principles and laws, and the Germans accumulated data.

Nevertheless internationalism was regarded as the birthright of science until such ideals were rudely shattered by the outbreak of the First World War. It has been said that Britain has habitually tended to be in a state of preparation for the previous war. Be that as it may, no attempt was made by the Government in 1914 to mobilize scientific talent in order to develop new and improved weapons. A wave of patriotic fervour swept the country

and scientists were able to volunteer for service in the armed forces without restriction. As a result many physicists were killed, Moseley being an outstanding example. Keller implies that this situation was remedied at the outset of World War Two, but those of us who suffered misdirection at the hands of the Joint Recruiting Board will have reservations on this point.

Something which may come as a surprise is the dominant role apparently played by the British Association for the Advancement of Science in providing a forum for the announcement of new discoveries and developments in atomic physics. In those days several of its annual meetings were held overseas, in Canada, Australia and South Africa, in spite of the long sea voyages this entailed for European participants. Nevertheless most leading scientists seem to have attended to hear of the latest results and ideas in original and often inspirational lectures delivered by such eminent figures as Crookes, J. J. Thomson,

Rutherford and Bohr. Nowadays, the function of the British Association seems to be more educational, bringing broad surveys of current scientific advances to wider, non-specialist audiences.

Included is a generous and candidly annotated bibliography, listing biographies of the principals, surveys of contemporary scientific advance and some modern historical studies of the period. Keller's comments are not only amusing and apposite, but they also indicate the shortcomings of the existing literature on his subject which he has so stylishly remedied in this book.

There are very few references in the text — no chapter has more than four. No doubt there will be some professional historians who cavil at this, but pay no heed to them. For this is a story to be read, enjoyed and savoured, not a definitive work of reference.

Few with a mature interest in the history of science are likely to forego this book. There is however another kind of potential reader who should be encouraged to acquire it, the younger practitioner of the physical sciences, who would thereby gain an awareness of what it must have been like to work in the laboratory alongside one of the founders of modern physics, in the days before huge machines came to dominate the field. □

Philip Davenport worked in the Clarendon Laboratory under Lord Cherwell and subsequently on the UKAEA Fusion Project, with which Sir George Thomson was closely associated. Now retired, he is an honorary consultant to Culham Laboratory on historical matters.

A map of the world

C.A. Russell

The Cambridge Illustrated History of the World's Science.

By Colin A. Ronan.

Newnes Books/Cambridge University Press: 1983. Pp.543. £12.95, \$29.95.

THIS bold enterprise, even if it is not quite as unusual as the publishers and author might like us to believe, is a remarkable compilation and outshines all its predecessors by its herculean attempt at comprehensiveness, in terms of space, time and theme. In 527 pages Ronan provides a commentary on the history of science from the magically inspired speculations of the ancient world to space science of the late twentieth century and from China to South America. It also includes 300 illustrations some of which have been rarely, if ever, produced in other recent books.

Quite inevitably in a work of such generality, one can spot errors of fact here and there (though comparatively few misprints). Samuel Wilberforce at the time of his confrontation with Huxley was Bishop of Oxford, not Winchester; Thomas Beddoes never occupied a Chair at Oxford; Davy's denial of the compound nature of chlorine rested upon other facts than electrolysis; Wöhler's synthesis of urea has been shown not to have the significance attributed to it; the mauve synthetic dye discovered by Perkin was not the same as the natural 'purple' of antiquity; the zero of Kelvin's absolute temperature scale was not the freezing point of water; Priestley is inaccurately described as a Presbyterian and so on. Were it only slips of this kind that called for comment the value of the book would not be seriously diminished. However it displays another tendency which might be deemed more important.

Partly on account of his desire to write engagingly for the layman, and doubtless also for reasons of space, the author frequently retails anecdotes in which fact and fiction are not always easily separable. He describes the exploits of Pythagoras, Archimedes and his bath, Galileo and the Tower of Pisa, Newton and his apple and much else in similar vein. When he cites Luther's alleged diatribe against Copernicus he gives no inkling of the scholarly doubts about the authenticity of the report on which it is based. His own dictum about Pliny: "the fabulous appears side by side with the prosaic" could at times be applied to his own narrative.

Recent historiography would also question some of the wider generalizations that are on offer. The 'timeliness' of Boyle's definition of an element is much to be doubted since no one did anything about it for over a century. There certainly was

doubt (and a great deal of it, especially in Germany) about the experimental evidence supporting Lavoisier's view of air. The role of science in the development of early steam power has been increasingly regarded with scepticism. And it is very doubtful if Kekulé could subscribe to a view of his own scientific strategy which presupposed a doctrine (valency) that he was himself later to discover! However it must also be stressed that there are many useful insights that are thoroughly up to date, most notable, perhaps, being Ronan's recognition of the inspiration due to animistic religion and the originality (as well as the conserving function) of medieval Arab science.

Perhaps the most remarkable feature of this book, and it is paradoxically both a virtue and a blemish, is its apparent freedom from ideological bias. Ronan really does seem to be trying to 'tell it like it is'. He ascribes the growth of science to neither the class war nor mystical intuition. He appears to have no stake in Puritanism, Islam, Zen Buddhism, or any of the other cults that have received much attention recently. Nor does he favour the 'heroic' view of history, and his participants emerge as far more ordinary men than many Victorians would have painted them. All this is a virtue. Yet, by playing it so straight down the line, the author leaves us with an uncomfortable sense of unreality.

The fact is that science did not just happen in a cultural vacuum, nor were all the 'scientists' (as they are often anachronistically called) operating in watertight compartments, their scientific and other interests having no effect on one another. To have any feeling of the

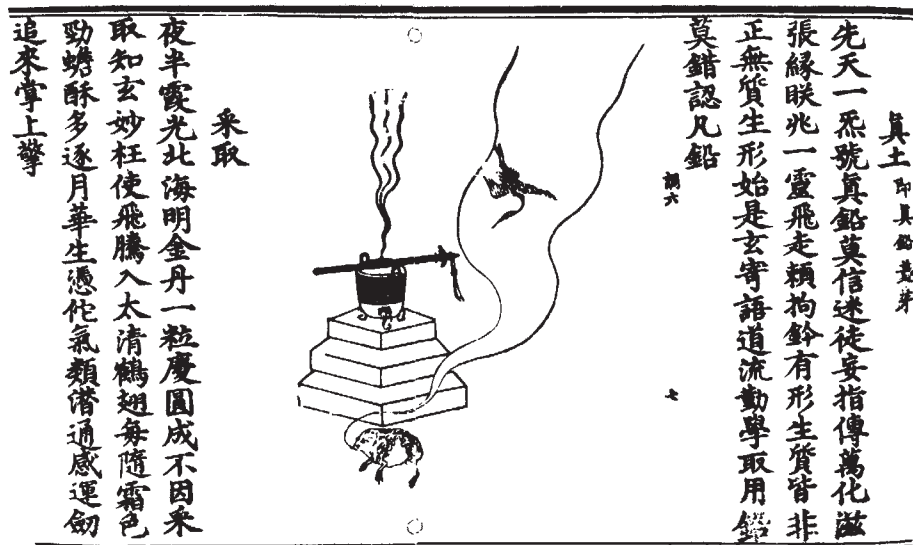
development of science we must know not only what happened but also why. For example, underlying the whole Darwinism debate were social and theological issues of immense importance and some complexity; but of these we get the merest hint (and, be it added, a very Victorian impression of a conflict that we now know to have been partly rigged at the time and largely misunderstood until quite recently). Again, Ronan writes of Newton's 'extraordinary theological interests' without apparently, any recognition of their immense relevance to all the science he ever did.

However there is an ideology behind the organization of the book, but it is not presented to us in those terms. Scientific progress is depicted throughout as something immensely desirable and probably inevitable. It is 'this glorious adventure of the human intellect'; despite reverses and setbacks, it is 'the long battle for the light'. In its positivist approach and 'Whiggish' flavour the book is reminiscent of a much earlier genre of historical writing.

Obviously this is not a book for the specialist. Reluctantly I conclude that it is not too reliable a guide for the novice who wishes to savour something of modern history of science. But for a general overview of how science emerged from its pre-history it will probably be most useful. If I earlier cited Ronan's words about Pliny and applied them to his own work, I should complete the quotation, for this is also appropriate: "The work is a magnificent compilation in spite of all its faults". □

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第六圖 金還丹印證圖



An illustration from a book by Lung Mei Tzu, dated to the 12th century, uses symbolism derived from early Chinese physiological alchemy. It appears in the latest volume of Joseph Needham's *Science and Civilisation in China* (Volume 5 *Chemistry and Chemical Technology* Part 5; *Spagyric Discovery and Invention: Physiological Alchemy*), which will be reviewed in a forthcoming edition of *Nature*.

Middle Age mortality

W.F. Bynum

The Black Death: Natural and Human Disaster in Medieval Europe.

By Robert S. Gottfried.

Robert Hall/Macmillan: 1983.

Pp.203. \$16.95, £9.95.

LEGIONNAIRES' disease, lassa fever, typhoid: despite our immunizations and antibiotics we remain psychologically vulnerable to epidemic disease, particularly if it is exotic or unexpected, or its cause is obscure. It could be, Robert Gottfried seems to suggest, that this nagging anxiety is just one more legacy of the Black Death, which he describes as 'the greatest biological-environmental event in history'.

Certainly the demographic, economic, social, political and cultural consequences of the European experience with plague during the three centuries following the Black Death of the 1340s were vast, even if Gottfried sometimes reaches for the hyperboles a bit too casually. Nevertheless, this latest biography of *Yersinia pestis* has much to recommend it.

There is, of course, no dearth of historical analysis of plague, most sweepingly perhaps as part of William McNeill's ambitious examination of the interplay of disease and history, *Plagues and Peoples* (Blackwell, 1976). Gottfried writes from the same ecological perspective as McNeill, and much the best part of his own work is the use he makes of recent research on climate, agricultural productivity, and population density in thirteenth and early fourteenth-century Europe.

He shows that even before the coming of plague, the medieval economy and social structure had been seriously affected by a century of cooler, wet weather and disastrous harvests. This provides a fascinating backdrop to his central chapters chronicling the beginnings, spread and immediate effects of the Black Death. While Gottfried is not so evocative as Philip Ziegler (*The Black Death*, Collins, 1969), his narrative holds the attention and his sober analyses of mortality estimates and social and psychological effects make compelling reading. Throughout Europe and the Middle East, the figures varied but the basic pattern was the same: 30-40% mortality in Pisa, as high as 60% in the villages surrounding Bury St Edmunds, "only" 10% in Nuremberg. In a sense, the wonder is that outbreaks of millenarianism, flagellation, or Jewish persecution were not more common than they were.

In another sense, though, the European experience with the Black Death demonstrates how resilient human beings can be, and there is a danger of reading too much back into this horrific episode and

the repeated outbreaks of plague during the next centuries. Gottfried is aware of this problem and reminds his readers occasionally that not *all* of European history between 1347 and 1666 can be seen in terms of the mentality and depopulation engendered by plague.

Too often, however, the caveats get lost in his rhetoric, as the plague is invoked as 'a' cause, or even 'the' cause, of virtually every major scientific, social, political or cultural transformation during the period. A good example of this tendency is his chapter entitled 'The Stirrings of Modern Medicine', in which a whole series of professional, educational and intellectual changes in medicine are associated with the Black Death: 'Before the Black Death. . . After the Black Death. . .'. These phrases

recur and Gottfried slips casually into this mode, sometimes sacrificing historical subtlety on the altar of dramatic effect. Consequently, the book requires thoughtful reading. But it is decidedly worth reading as a stimulating introduction to its topic and a cogent synthesis of modern scholarship on plague. That scholarship itself can be approached by Gottfried's helpful 'Bibliographical Essay' (pp. 187-194), the extent of which testifies to the continuing historical preoccupation with study of the impact of epidemic disease on our ancestors and thereby on ourselves. □

Dr. W.F. Bynum is at the Wellcome Institute for the History of Medicine, London.

Diet and decline

Alex Comfort

Handbook of Nutrition, Health and Ageing.

By Donald M. Watkin.

Noyes Publications, Park Ridge, New Jersey: 1983. Pp.290. \$32.

A HANDBOOK is a comprehensive practical summary. So a handbook of nutrition, health and ageing might be expected to consist of more than one volume. At the same time, while public pressure and some dedicated missionary activity have not yet succeeded in raising geriatric medicine in the United States to European levels, or in mobilizing proper social services to support it, they have persuaded publishers to add the word "ageing" to as many titles as possible — often with little change in the matter which follows.

This, unfortunately, is the case with Dr Watkin's book. Nutrition is a hard subject to address because, as in economics, the experts and the results are often at odds. What Dr Watkin has compiled is in essence a double book. The first section is an elementary summary of nutrition, the second a useful though short survey of Federal nutrition programmes. Over these, the issue of ageing has been scattered, as from a pepper-grinder: one is glad to see it there, but sorry that existing research in this field has not been more systematically addressed.

I find this extraordinary, because, as Dr Watkin points out, caloric restriction is at the moment the only experimental intervention which appears to modify the rate of senescence in mammals. Other nutritional maxims attacking excessive consumption of fats, unbalanced diets and alcohol are aimed at causes of shortening life, the substance of these has changed remarkably little since Cornaro or Hufeland, though Dr Watkin presents them in more fashionable dress. In a

section headed "State of the Art", Watkin cites McCay, mentions a couple of the wide-ranging and important papers by Ross and then reports his failure to find any further relevant studies on Medlars — hardly surprising, since the focus in investigating the mechanism of the McCay effect has shifted to neuroamines, immunology and other areas: the "state of the art" seems to have slipped through Dr Watkin's net.

In the area of health-maintenance he does rather better; the bibliography would be useful if it were alphabetically arranged. Dr Watkin has the conviction — probably correct — that much could be done through nutrition research to reduce age-linked disability (an attack on alcohol and cigarettes would certainly do this) but the two brief sections on the "State of the Art" (12 pages) and "Putting it All Together" (10 pages) hardly live up to their promise. They give the impression that the Federal programmes which Watkin summarizes, and which are admirable as far as they go, have the matter under control — which they haven't. Not only has nutrition research in preventive medicine a sizeable Augean stable to cleanse before it reaches convincing unanimity: with malnutrition due to poverty and cutbacks in social programmes a growing problem in Reaganomic America, the book gives an over-rosy picture of a contentious, disorderly and partisan field of investigation — one where what science there is, is frustrated by politics.

A comprehensive review of nutrition in relation to gerontology and geriatrics remains to be written. A comprehensive review of nutrition and health in the underfed could now be written from abundant experience, unfortunately. A comprehensive review of nutrition in the overfed and injudiciously fed is badly needed but its time has not yet come. America contains both populations, and both of them are ageing. □

Alex Comfort is an Adjunct Professor at the University of California, Los Angeles.

Chance and necessity

Michael Berry

Regular and Stochastic Motion.

By A.J. Lichtenberg and M.A. Lieberman.

Springer-Verlag: 1983.

Pp. 500. DM 108. \$44.60.

IN RECENT decades the study of classical mechanics has revived, to the point where it is no exaggeration to claim that the subject is in the full flood of a renaissance. The new developments contain the resolution of an apparent contradiction which puzzled many physicists and mathematicians since Newton.

Consider the motion of a particle acted on by forces. Sometimes the motion is predictable ('regular'), as in the Keplerian motion of planets whose mutual gravitation is neglected, and sometimes the motion is unpredictable (chaotic or 'stochastic'), as in a pinball machine. Now we are learning that between these extremes of 'necessity and chance' or 'fate and luck' lie orbit structures with a marvellous richness: for a typical system of forces, some orbits are regular and some are stochastic, depending on initial conditions, and the different types can be intimately mixed like rational numbers among real numbers. Such discoveries must have methodological implications for those sciences which take Newtonian mechanics as their paradigm.

Many research papers have been published and a few general review articles have been written, with the result that the elements of the New Mechanics are becoming widely known. But what has been lacking is a 'workshop manual' in which the techniques are presented in detail from the beginning. This is exactly what Lichtenberg and Lieberman provide and it is very welcome.

The main strength of the book is its comprehensive discussion of how stochasticity grows when a system whose motion is initially regular is subjected to perturbations of increasing strength. Stochastic

ity sneaks in through resonances, which correspond to periodic orbits of the unperturbed system. The resonances cause classical perturbation theory to be far more complicated than its quantum-mechanical counterpart. Nevertheless, 'super-convergent' schemes show via the celebrated Kolmogorov-Arnold-Moser (KAM) theorem that regular motion does survive perturbation, at least until there is widespread overlap of resonances.

Complicated motion does not require the interaction of many particles (as in a gas) but can occur even for a single particle moving in two dimensions under the action of external forces. For three or more degrees of freedom, however, a qualitatively new feature occurs (the Arnold diffusion) and the thorough discussion of this is another strong point.

The formal developments are abundantly illustrated by examples, whose physical backgrounds are sketched in an appendix. These include: stability of planetary

motion, particle accelerators, magnetic confinement and chemical dynamics.

I have a few minor criticisms. The important fact that in regular motion the orbits explore tori in the system's phase space is introduced rather late and not emphasized. This leads to a confusion between the concepts of integrability and separability. It is recognized that strange attractors have a hierarchical structure describable in terms of a fractal dimension and yet, unaccountably, the work of Mandelbrot is nowhere mentioned. There is little use of the modern notation of differential forms to simplify phase-space (symplectic) geometry.

In spite of these criticisms I do strongly recommend this book to those who are contemplating or carrying out research in modern mechanics and who need to learn the nuts and bolts of the subject. □

Professor Michael Berry is in the H.H. Wills Physics Laboratory, University of Bristol.

Boundary matters

D.H. Everett

Colloid and Interface Chemistry.

By Robert D. Vold and Marjorie J. Vold.

Addison Wesley: 1983. Pp 692.

£63.70, \$137.50.

THERE has long been a need for a comprehensive modern book on colloid and interface chemistry. Kruyt's outstanding *Colloid Science* is now over thirty years old, and during this time enormous advances have been made in both the experimental investigation and in the understanding of colloid phenomena.

Robert and Marjorie Vold are among those who have contributed substantially to these advances so that one hoped that their book would fill this important gap in the scientific literature. Alas, in my opinion, that gap still remains. This is a particularly unhappy conclusion, reached reluctantly, as Robert's untimely death prevented him from collaborating in the final stages of publication.

The book is for "everyone interested in surface and colloid science", but mainly for graduate students, with ambitious undergraduates and experienced practitioners as secondary beneficiaries. The breadth and depth of the subject is emphasized by the reply given to the question "How long does it take to master colloid and interface chemistry?" "More than a lifetime." Perhaps no one, or even a pair of authors, should attempt to cover the whole field. The present authors have attempted, and to some degree succeeded in giving a broad perspective, but the book contains many errors, and the treatment of several subjects is open to serious criticism.

In many instances, a precis of an authoritative article or book is given with

the comment that further details are to be found in the original. Too often, these omitted details include some which are absolutely essential to the argument, so that the book cannot be used properly by itself nor understood fully without consultation of the literature cited.

Perhaps the weakest aspect of the book is the thoroughly unsatisfactory use of thermodynamics. The phraseology is loose and definitions are often ambiguous. Adsorption is defined in confusing terms and no justification is given for including surface terms in the equation for the energy of a system containing an interface. How an algebraic manipulation of three equations — none of which includes any surface terms — can lead to an equation including a surface contribution is a mystery. And the identification of surface tension with 'surface free energy' without any qualification will lead to the perpetuation of a misunderstanding which one had hoped had been eliminated years ago.

This book seems to have been constructed around a comprehensive set of lecture notes. General statements, a certain amount of hand-waving, and the occasional quip, may be justified in a lecture, when backed up by a reading list. But in a textbook one expects a much greater degree of precision, which is so essential in such a complex subject as colloid science. The misunderstandings which could arise from an uncritical reading of this book would be clarified by judicious use of the extensive references, but it is a great pity that a more rigorous approach has not been adopted, and that the underlying principles of colloid science do not shine through as clearly as they should. It is with considerable regret that I find myself unable to give this book my unqualified commendation. □

D.H. Everett is Emeritus Professor of Physical Chemistry at the University of Bristol.

Journals review issue 1983

New week *Nature* will publish the third review supplement devoted to new science journals. The two previous supplements (covering journals first published between January 1978 and May 1980 and June 1980 and May 1981) appeared in *Nature* 293, 341-369 (1981) and 299, 491-514 (1982).

Journals appearing between June 1981 and May 1982 and earlier journals not covered in previous issues, have been considered.

Appointments

A total of 36 new members have been elected to the American Institute of Medicine, raising the active membership to 456. In addition, five people have been directly elected to senior membership, bringing the total number of senior members to 194. The following are the newly-elected members: **Dr Ronald Andersen**, (Center for Health Administration Studies and Graduate Program in Hospital Administration, University of Chicago, USA); **Professor Howard Ballit**, (School of Public Health, Columbia University, New York City, USA); **Professor James Bawden**, (Dental Research Center, University of North Carolina, Chapel Hill, USA); **Dr Steven Beering**, (Purdue University, West Lafayette, Indiana, USA); **Dr Allan Beigel**, (Southern Arizona Mental Health Center, Tucson, Arizona, USA); **Professor George Benedek**, (Massachusetts Institute of Technology, Cambridge, Massachusetts, USA); **Dr J. Robert Buchanan**, (Massachusetts General Hospital, Boston, Massachusetts, USA); **Dr William Carey**, (Media, Pennsylvania, USA); **Professor Purnell Choppin**, (The Rockefeller University, New York City, USA); **Professor Peter Dews**, (Harvard Medical School, Harvard University, Boston, Massachusetts, USA); **Professor Rhetaugh Dumas**, (School of Nursing, University of Michigan, Ann Arbor, Michigan, USA); **Professor Mitzi Duxbury**, (School of Nursing and Center for Health Services Research, University of Minnesota, Minneapolis, Minnesota, USA); **Dr David Eddy**, (Center for Health Policy Research and Education, Duke University, Durham, North Carolina, USA); **Dr Charles Edwards**, (Scripps Clinic and Research Foundation, La Jolla, California, USA); **Dr Edward Evarts**, (National Institute of Mental Health, Bethesda, Maryland, USA); **Monsignor Charles Fahey**, (School of Social Studies, Fordham University, New York City, USA); **Professor Howard Freeman**, (University of California, Los Angeles, California, USA); **Dr Jerome Grossman**, (New England Medical Center, Boston, Massachusetts, USA); **Professor Melvin Grumbach**, (University of California, San Francisco, California, USA); **Dr Curtis Hames**, (Claxton, Georgia, USA); **Professor Edward Hook**, (University of Virginia, Charlottesville, Virginia, USA); **Professor Lyle Jones**, (University of North Carolina, Chapel Hill, North Carolina, USA); **Professor Robert Katzman**, (Albert Einstein College of Medicine, Montefiore

Medical Center, Bronx, New York, USA); **Professor Stuart Kornfeld**, (School of Medicine, Washington University, Pullman, Washington, USA); **Professor Paul Lacy**, (School of Medicine, Washington University, Pullman, Washington, USA); **Dr Claude Lenfant**, (National Heart, Lung and Blood Institute, National Institutes of Health, Bethesda, Maryland, USA); **Mr Lawrence Lewin**, (Lewin and Associates, Inc., Washington, DC, USA); **Dr C. S. Lewis**, (Tulsa, Oklahoma, USA); **Professor Hugh McDevitt**, (Stanford University School of Medicine, California, USA); **Dr James Pittman**, (School of Medicine, University of Alabama, Birmingham, Alabama, USA); **Professor Charles Rosenberg**, (University of Pennsylvania, Philadelphia, Pennsylvania, USA); **Dr Louise Russell**, (The Brookings Institution, Washington, DC, USA); **Dr Bruce Jones Sams**, (The Permanente Medical Group, Inc., Oakland, California, USA); **Professor Robert Schimke**, (Stanford University, California, USA); **Professor Margretta Styles**, (School of Nursing, University of California, San Francisco, California, USA); **Professor Sheldon Wolff**, (Tufts University School of Medicine, Medford, Massachusetts, USA). Those elected to senior membership are: **Dr Tibor Greenwalt**, (Hoxworth Blood Center, University of Cincinnati Medical Center, Cincinnati, Ohio, USA); **Professor Robert Hofstadter**, (Stanford University, California, USA); **Dr Moshe Prywes**, (University Centre for Health Sciences and the Centre for Medical Education, Ben Gurion University of the Negev, Beer Sheva, Israel); **Mr Malcom Randall**, (Veterans Administration Medical Center, Gainesville, Florida, USA); **Dr John Seal**, (formerly of the Office of the Director, National Institute of Health, Bethesda, Maryland, USA).

Two new members have been appointed to the Science and Engineering Research Council. They are **Professor Richard Norman**, chief scientific adviser to the British Ministry of Defence and **Derek Colley**, professor of high energy physics at Birmingham University, UK.

Four new members have been appointed to the Natural Environment Research Council and two other members have been re-elected. The new members are **Professor Robert Clark**, **Professor Richard Cormack**, **Professor John Dewey** and **Mr Ferdinand Larminie**. They replace Professor John Allen, Professor Michael Audley Charles, Professor Sir James Beaumont

and Mr George Williams, who are retiring. The two reappointed members are **Professor James Briden** and **Professor John Monteith**.

The American Society for Microbiology (ASM) and the Burroughs Wellcome Fund have selected three Wellcome visiting professorships in microbiology for 1983-84. The following have been named as recipients: at Indiana University, **Professor Eugene Nester**, currently at the department of microbiology and immunology of the University of Washington School of Medicine, USA; at the University of Texas Health Science Center at San Antonio, **Professor David Botstein**, currently at the Massachusetts Institute of Technology, USA; at the University of South Carolina, **Professor Thomas Merigan**, who is currently at the Stanford University School of Medicine, California, USA.

The British Institution of Electronic and Radio Engineers has announced that **Professor David Davies** is to be its next president.

Dr Martin Downer has been appointed chief dental officer at the Department of Health and Social Security, UK. Dr Downer is at present chief dental officer at the Scottish Home and Health Department.

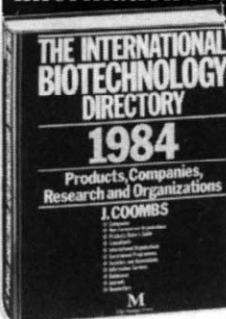
Mr Maldwyn John has been made president of the British Institution of Electrical Engineers (IEE).

Awards

Professor Kenneth Murray of the department of molecular biology at the University of Edinburgh has been awarded the Willem Meindert de Hoop prize for his work on recombinant DNA technology and on hepatitis. The prize carries an award of 50,000 Dfl (\$17,000).

The council of the British Royal Society has made the 1983 Royal Society Esso energy award to **Mr J. Masters** and **Mr R. J. Webb** of the British Gas Corporation research and development division for their development of a recuperative burner system for gas-fired furnaces. The Esso award consists of a gold medal and a prize of £1,000 and is given annually for an outstanding contribution to the advancement of science, technology or engineering that leads to the more efficient mobilization, use or conservation of energy resources.

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Miscellaneous

A trust fund has been set up to enable young scientists to attend the forthcoming International Biophysics Congress to be held in Bristol, UK, from 29 July to 4 August 1984. The fund is being run by the International Union of Pure and Applied Biophysics (IUPAB) and the Royal Society. Further information may be obtained from Dr H. C. Watson, Secretary General of IUPAB, Department of Biochemistry, University of Bristol, Medical School, University Walk, Bristol BS8 1TD, UK).

The Scripps Clinic and Research Foundation has announced the establishment of the W. M. Keck Foundation Autoimmune Disease Center. Research carried out in the centre will cover the causes and treatment of autoimmune diseases such as rheumatoid arthritis and systemic lupus erythematosus (a generalized connective tissue disorder primarily affecting women in mid-life). The centre will be under the direction of Dr Eng Tan.

The British Library has granted a licence to Chadwyck-Healey to publish the first index to provide a listing of the holdings of the department of manuscripts in one alphabetical sequence. The first volume of the index will be published in the spring of 1984, with publication scheduled to be completed a year later. It will be in 30 library-bound volumes of some 800 pages each, containing approximately 400,000 entries.

The British Institution of Electronic and Radio Engineers has formed a specialist electromagnetic compatibility group. Amongst the topics that the group will look at are biological hazards, lightning and static electricity, coupling within systems down to component level and nuclear effects. The group has assumed responsibility for continuing the international conferences on electromagnetic compatibility, the next of which will be held at the University of Surrey, UK, from 18 to 20 September 1984. Further information is available from The Secretary, EMC Group Committee, Institution of Electronic and Radio Engineers, 99 Gower Street, London WC1E 6AZ, UK.

Entries are invited for the annual Ray Bruner science writing fellowship which is run by the American Public Health Association. The fellowship is open to any journalist with less than two years' experience in the health/science/medical field and with no more than five years' full-time reporting experience. Entries must be received by 14 October 1983. The winner will be invited to cover the American Public Health Association's annual meeting. The round-trip air fare will be covered, and a

\$500 prize will be awarded. Applications should be sent to the Ray Bruner Science Writing Fellowship, Attention: Doyné Bailey, American Public Health Association, 1015 15th Street, NW, Washington, DC 20005, USA.

Meetings

4-6 January, **Biointeractions '84**, London (Ms M. Korndorffer, Conference Organizer, Biomaterials, PO Box 63, Westbury House, Bury Street, Guildford, Surrey GU2 5BH, UK).

16-20 January, **Miami Winter Symposium; Advances in Gene Technology: Human Genetic Disorders**, Miami Beach, Florida (Miami Winter Symposia, PO Box 016129, Miami, Florida 33101, USA).

20 January, **Joint Meeting of the Royal Society of Medicine, the British Institute of Radiology and the Royal College of Radiologists**, London (Mrs. J.M. Royston, The British Institute of Radiology, 36 Portland Place, London W1N 3DG, UK).

20-24 February **Chapman Conference on Collisionless Shock Waves in the Heliosphere**, Napa Valley, California (R.G. Stone, Code 690, NASA/Goddard Space Flight Center, Greenbelt, Maryland 20771, USA).

29 February, **Information Retrieval of Environmental Chemicals**, London (Dr C.S. Collis, 46 Lyndhurst Road, London N22 5AT, UK).

5-9 March, **Pittsburgh Conference and Exposition on Analytical Chemistry and Applied Spectroscopy**, Atlantic City, New Jersey (Mr G.L. Vassilaros, Exposition Chairman, Pittsburgh Conference, 437 Donald Road, Department CFP, Pittsburgh, PA 15235, USA).

6-9 March, **Oceanology International 84**, Brighton (Spearhead Exhibitions, Rowe House, 55-59 Fife Road, Kingston upon Thames, Surrey KT1 1TA, UK).

12-16 March, **Lunar and Planetary Science Conference**, Houston (Ms P. Jones, Conference Administrator, Lunar and Planetary Institute, 3303 NASA Road 1, Houston, Texas 77058, USA).

15-16 March, **International Symposium on Calcium Entry Blockers and Tissue Protection**, Rome (Organizing Secretariat, International Symposium on Calcium Entry Blockers and Tissue Protection, Institute of Pharmacology and Pharmacognosy, Via Balzaretti 9, 20133 Milan, Italy).

19-23 March, **International Seminar on the Offshore Mineral Resources**, Brest, France (Mr L. Galtier, Association Germinal, BP 6009, 45060 Orléans Cedex, France).

21-23 March, **Meeting on Surface Active Agents**, Tenerife (XV Jornadas del Comité Espanol de la Detergencia, Secretaria de la Asociacion de Investigacion de Detergentes, c/o Jorge Girona Salgado, s/n-Edificio Juan de la Cierva, Barcelona-34, Spain).